DISEASES ACQUIRED FROM MICROBIOLOGICALLY CONTAMINATED AIRCONDITIONING SYSTEMS

BY

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ABSTRACT

An account of the design and function of airconditioning systems is provided in order to lay the foundation of study for the non-engineer. Certain components, particularly those containing large bodies of warm water, are prone to contamination with microorganisms. Such components are described because they present a hazard to the health of the building's occupants. Methods for collecting and handling specimens from these sources are included.

Droplet nuclei less than 4.5um in diameter are the most likely particles to cause disease. They are distributed via the airhandling plant of the airconditioning system to all areas of the building. Particles of this size are able to evade the clearance mechanisms of the lung and be deposited in the finest airways where they can cause disease.

There are two major diseases acquired from microbiologically contaminated airconditioning systems. Legionnaires' disease manifests as pneumonia and is caused by a gram-negative bacillus, the type species of which is Legionella pneumophila. Hypersensitivity pneumonitis is an allergic disease resulting from the inhalation of organic material. Lung dysfunction and a variety of generalized symptoms occur.

Recommendations are made concerning the prevention of diseases from contaminated airconditioning systems and an approach is suggested towards their investigation.

INTRODUCTION

In 1976, 182 delegates attending an American Legion's conference developed a febrile illness, later known as Legionnaires' disease. There were 29 deaths. This outbreak brought to the public's attention the involvement of airconditioning systems in disease. Indeed, many engineers and microbiologists were similarly enlightened.

The discovery of a new disease caused by an organism previously unknown to man sparked great interest amongst microbiologists. As a result, intensive research rapidly produced information covering all aspects of the disease. Details concerning the epidemiology demonstrated that in many outbreaks an airconditioning system was involved.

It was this information that directed the field of study for this thesis towards diseases acquired from microbiologically contaminated airconditioning systems. An examination of the literature showed that there were other diseases which fitted into this category, notably hypersensitivity pneumonitis and the related condition, humidifier fever, neither of which has received much publicity. Despite the fact that millions of people work in airconditioned buildings few people seemed to be aware of the possible hazards. It became evident that, in general, the medical profession was unfamiliar with the symptoms of these diseases (Marinkovich V.A., Hill A., 1975), and microbiologists were unable to recommend appropriate specimens that should be collected nor were they aware

of the techniques available to process them. Engineers were guilty of poor design increasing the likelihood of contamination and maintenance personnel failed to understand the importance of preventative techniques (Friend J.A.R., Gaddie J., Palmer K.N.V., 1977; Walter C.W., 1966).

It would appear that the lack of awareness to problems concerning microbiologically contaminated airconditioning systems needs to be redressed. It is the aim of this thesis to review the literature, principally from the position of a microbiologist, but also that of an engineer with the objectives of providing information and considering preventative measures.

In order to understand the relationship between the airconditioning system and disease, it is necessary to commence with an examination of the apparatus involved, for it is at this level that contamination occurs. The apparatus can be considered to be the focal source of the disease in the ecosystem of the building. The first chapter of the thesis covers this subject, following which, the link between the source and the susceptible host has to be made. This involves a number of separate steps. Firstly, airflow has to be considered as air is the vehicle of transmission. Because one of the functions of the airconditioning system is to distribute air, this route is particularly effective. Microorganisms are spread on dust particles, in water droplets or as droplet nuclei. The question is, are they all capable of causing disease or is one mode of spread more significant than the others? The final stage in this process of infection is the

contact of the particle with the host in such a way that disease results. This area too is considered.

It is through the investigation of this sequence of events that it was hoped to reveal a plan for control of the spread of disease. Failing that, would it be possible to identify those workers at risk of acquiring disease? It had already been determined in outbreaks of Legionnaires' disease that only a small percentage of people exposed to the organism progressed to the disease state. Was there an explanation for this?

One of the major aims of this project was to assimilate information that could be used to develop a masterplan for investigating contaminated airconditioning systems. In order to achieve this, specimens need to be collected followed by various laboratory techniques such as concentration, isolation and identification of microorganisms. Whilst it is beyond the scope of this thesis to detail these techniques, references are presented which allow the reader to pursue specific areas in more detail.

A detailed description of the two major diseases associated with contaminated airconditioning systems, Legionnaires' disease and hypersensitivity pneumonitis, is presented. Included, is the history, clinical features and diagnosis, pathology, laboratory methods, treatment, epidemiology and control aspects. It is acknowledged that reviews already exist covering these diseases, however, there is room for recent developments to be included, a different perspective

to be placed on the information and other material to be added. In some instances, confusion has been created by conflicting reports and variations in nomenclature. As far as possible these areas have been clarified.

A decade has elapsed since Legionnaires' disease was first described and even longer for hypersensitivity pneumonitis. However, outbreaks of both diseases still occur with fatal consequences. A recent outbreak in South Australia attests to this (see newspaper article). Are these cases avoidable? Would a greater understanding of the prevention and diagnosis have reduced their incidence? These are the questions that will be considered in this thesis.

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Legionnaires outbreak in Adelaide

ADELAIDE — South Australian health authorities are investigating an outbreak of legionnaires disease in Adelaide's southern suburbs which has claimed two lives and possibly a third.

The acting director of the Public Health Service, Dr Chris Baker, said yesterday that there had been 12 confirmed cases of the disease and 10 possible cases.

They had all occurred between December 22 last year and January 10 this year and investigations were under way to determine the source of the infection.

The outbreak was known to be responsible for two deaths and health officials were investigation the possibility it was also responsible for a third.

Dr Baker said it appeared exposure of victims to the legionnaires organism occurred over a very short time, possibly just one day, and was confined to a small area, with a cluster of cases in suburban Daw Park.

He said it was too early to speculate on a precise source of the infection, although a spokesman for Health Minister John Cornwall admitted the Daw Park Repatriation Hospital was one of a number of sites under investigation.

Dr Baker said it appeared the outbreak was now over as no cases had been reported since January 10.

Before this current outbreak, legionnaires disease claimed 10 lives from 30 reported cases in South Australia between 1982 and 1985.

Symptoms of the disease, which was first isolated in America in 1978, include loss of appetite, muscle aches, headaches, and fever with chills.

A significant proportion of the population come in contact with the infection and many carry legionnaires anti-bodies, but it is uncommon for them to develop symptoms.

The legionnaires organism often circulates in air-conditioning systems.

CHAPTER 1

COMPONENTS OF AIRCONDITIONING SYSTEMS

The following account of design and engineering aspects of airconditioning systems is given in order to describe the individual components, their function and mode of operation. The main purpose of ventilation and airconditioning systems is to distribute warmed or cooled air throughout the building. In so doing, this provides a means for the airborne transmission of any contaminants which may be present (Ager B.P., Tickner J.A., 1983).

In chapter 2, those components that may act as reservoirs of microorganisms will be identified and discussed in more detail.

Mechanical systems for the supply and distribution of fresh air are present in most industrial, commercial and institutional buildings. Often the system is designed to accomplish specific conditions, such as filtering, heating, cooling or humidifying as required (Ager B.P., Tickner J.A., 1983). Ideally, airconditioning systems should provide a comfortable environment free from temperature variation, poor ventilation, draughts and odours (ACOA Report, 1984).

Temperature

Air is heated or cooled in order to provide conditions for the occupants such that minimal energy be expended to perform their duties. The recommended air temperature is

a range, set according to fluctuations in the outside seasonal air changes. Accordingly, this will vary from month to month.

Recommended office air temperature =

(mean monthly temperature x 0.31) + 17.6°C

(ACOA Report, 1984).

Generally, however, the range of air temperature that is comfortable for most people is 21°C - 26°C (ACOA Report, 1984).

Air Movement and Circulation

The nature of ventilation requirements and acceptable limits are not clearly defined. Low flow rates of 1 metre^3/ hour will provide sufficient oxygen but body odour may become objectionable. Much larger requirements, approaching 40 metres^3/hour reduce the level of odour to permissible levels (17th symposium, 1967).

In America recommendations include the following minimum air changes of outdoor air per hour: four changes for operating rooms; three for trauma, delivery and cardiac catheterization rooms; and one air change per hour for nurseries. Total air change requirements (outdoor air plus recirculated air) are also recommended, for example, two air changes per hour for patients rooms, six for isolation rooms and 20 for operating rooms (Mallinson G.F., 1984).

Table I

Ventilation requirements in hospitals, nursing homes and convalescent homes (Australian Standards 1668, 1980)

Class of occupancy	Floor area person/metre^2	Minimum fresh air requirement per person. Litres/sec
Foyers	2	10
Hallways	2	10
Bedrooms	6.6	5
Wards	5	5
Food service centres	5	7.5
Operating/Delivery rooms	-	10
Amphitheatres	1	5

Humidity

A relative humidity of 40-50% is regarded as ideal for human comfort, however, at 22^{o}C dry bulb temperature, this degree of humidity would result in condensation on windows and inner surfaces. Consequently, the practical relative humidity at 22^{o}C is 30% (Harris N.C., Conde D.F., 1974). Relative humidities of less than 30% can dry mucous membranes and may increase risk of infection. It is, therefore, preferable to maintain humidities at least this high with periodic checks using a sling psychrometer (Mallinson G.F., 1984).

In warm outside conditions, air usually contains high humidity with the opposite being true for cold temperatures. It is necessary to either dehumidify or humidify respectively, before allowing air to enter the conditioned space (Harris N.C., Conde D.F., 1974).

Reclaiming Energy

In some buildings it is possible to conserve energy by mixing returned air with fresh air in a plenum, before conditioning and recirculating. Air from the plenum is filtered to remove airborne particles such as dust, smoke, microorganisms and pollen (Croome-Gale D.J., Roberts B.M., 1975). Another method that is used in hospitals is to reclaim the energy that is discharged as exhausted air. Two systems are available, one is a circulating 'heat wheel' and the other is a coil with circulating water. Either system is positioned so that it removes heat or cold from an exhaust-air duct for transfer to a supply-air duct (Mallinson G.F., 1984).

Components of an Airconditioning System

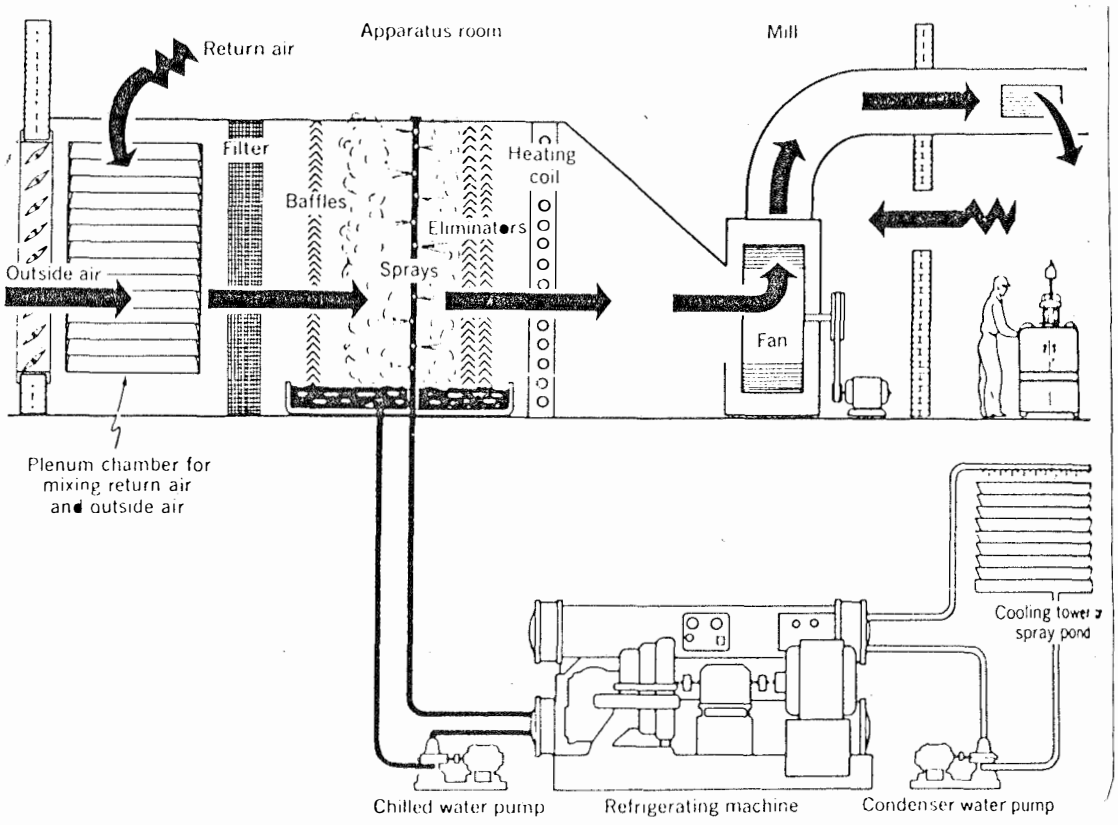
Airconditioning equipment includes, fans to move air, ducting to convey air and grills and diffusers to distribute air. Plant items include: filters for aircleaning; coils and spray apparatus for cooling and dehumidifying; various humidifiers; dampers for air volume and mixing; a refrigeration plant; condensing equipment; boilers and

heat exchangers; circulating pumps and pipework (Harris N.C., Conde D.F., 1974).

Figure 1

Components of basic airconditioning systems

(Harris N.C., Conde D.F., 1974)



The equipment can be divided into four classifications:

i) Heating and non-evaporative cooling systems

ii) Water-dependent air cooling, washing and humidifying devices

iii) Evaporative water cooling equipment

iv) Air conveying equipment.

Heating and Non-evaporative Cooling Systems

Air may be heated directly through an electric heating element or indirectly using a hot water boiler. The boiler produces water under pressure at a temperature of approximately 104°C to the hot water coil. Heat is transferred to the air to be conditioned via the hot water coil (Harris N.C., Conde D.F., 1974).

Air is usually cooled through an indirect mechanism such as a cooling coil (ACOA Report, 1984) however, refrigerant machinery is also used. In this case, a compressor driven by an electric motor is attached to an air cooled condenser. The compressor uses freon 22 as a refrigerant which is supplied to the cooling coil. The refrigerant boils at 4.4°C thus removing heat from the air by exchange. This coil also acts as a dehumidifier in summer with moisture in the air condensing on the cold coil (Harris N.C., Conde D.F., 1974).

Water-Dependent Air Cooling, Washing and Humidifying Devices

Transfer of both heat and water vapour can be achieved with a spray washer. This apparatus is common to many air-conditioning systems in Australia (ACOA Report, 1984) and brings air into intimate contact with water-sprays which use water recycled from a storage bath. The spray water can be heated to improve humidification. Airborne particles may be removed by the washing action of the sprays (Croome-Gale D.J., Roberts B.M., 1975).

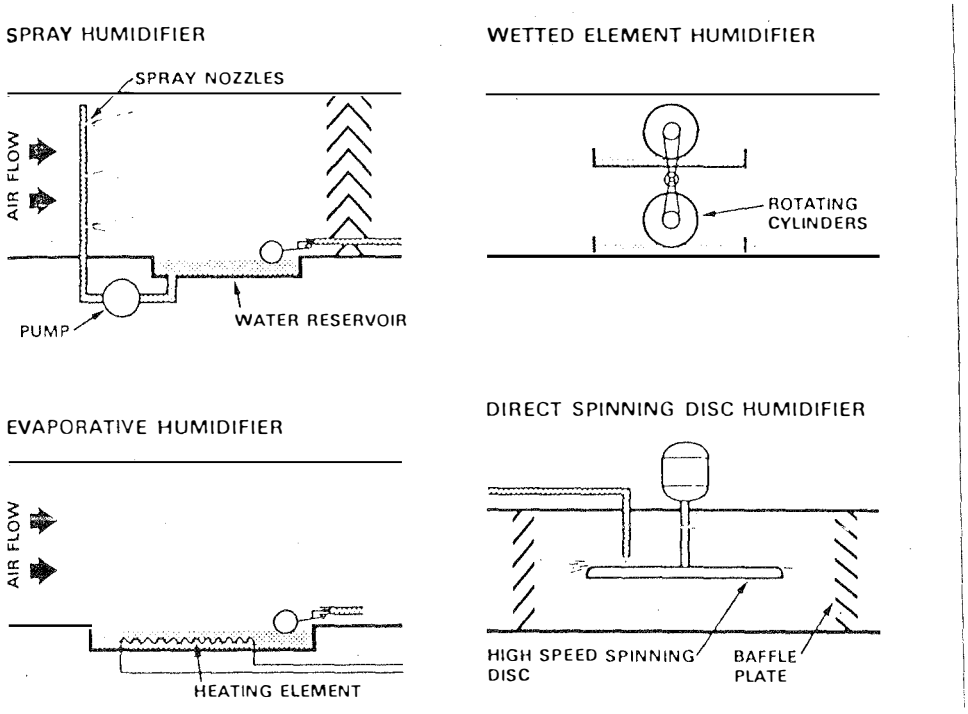
Humidifiers

Humidification of an air stream is achieved by either evaporation of water or steam injection. Dry steam is injected into the moving air stream. This method removes the problems of pipe corrosion, bacterial growth and odour production (Croome-Gale D.J., Roberts B.M., 1975).

In humidifiers in which atomizing sprays are used to promote intimate contact between air and water, it is necessary to provide some means of preventing large droplets from being discharged. Baffle plates or 'eliminators' are fitted for this purpose. Water temperatures in this apparatus rarely exceed 20°C (Ager B.P., Tickner J.A., 1983).

Figure 2

Common types of humidifiers (Ager B.P., Tickner J.A., 1983)



Evaporative Water Cooling Equipment

Refrigeration plant and airconditioning systems extract considerable quantities of heat. As these systems cannot store heat it must be removed at the rate at which it is extracted. This is achieved through exchanging heat, usually to the external atmosphere (Ager B.P., Tickner J.A., 1983). Heat exchanging apparatus usually employ large quantities of water. As such they are often sited on top of buildings.

Spray ponds consist of a basin for water collection with water piping above. Water is sprayed upwards, coming into contact with air and being cooled by it (Harris N.C., Conde D.F., 1974).

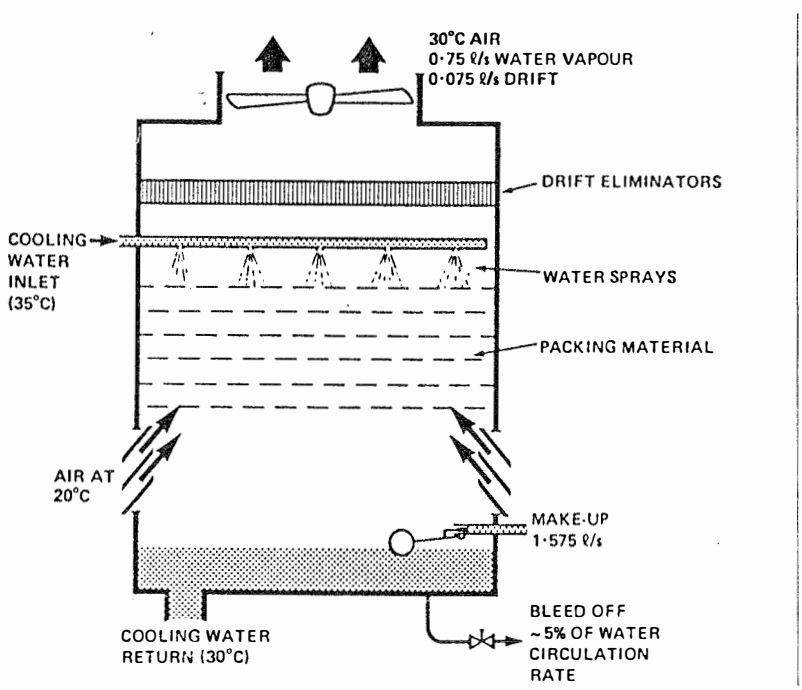
There are two types of cooling towers. One, a spray filled tower, consists of a system of spray nozzles which sprays warm water to a basin 2.5-3 metres below. This structure is surrounded by louver-board walls which are continuously wetted in order to maximise the water surface exposed to air. The other kind of tower is deck-filled and may be a huge structure. Warm water trickles over many decks until it reaches the bottom. Unlike the spray pond and spray-filled tower which require a wind velocity of 5-8 kph to obtain efficiency, the deck-filled tower may operate at zero wind velocity (Harris N.C., Conde D.F., 1974). Some towers rely upon a forced circulation of their own to provide evaporative cooling. Water trickles

over the decking from the top of the tower with fans providing the air flow (Ager B.P., Tickner J.A., 1983).

Figure 3

Schematic of a typical cooling tower

(Ager B.P., Tickner J.A., 1983)



Air Conveying Equipment

The supply air fan draws in fresh air from outside the building where it may be combined with return air in the mixing chamber or plenum. The air is then drawn through filters where dust and other foreign matter is removed. The filtered air is then heated, cooled and humidified as required before being blown through the ductwork and distributed into the airconditioned space.

If the system recycles conditioned air then between 80% to 90% will be recirculated. The remainder will be exhausted from the building through relief air grilles (ACOA Report, 1984).

Ducting is usually constructed from galvanised iron sheets, although aluminium, plastics and fibreglass are receiving more common usage. Baffles may be situated at junctions and bends to improve airflow and reduce noise and vibration (Croome-Gale D.J., Roberts B.M., 1975).

Filters are usually fitted after the air intake and before the fan of the supply air system. A coarse pre-filter is often used followed by a finer after-filter (ACOA Report, 1984). The contaminants that may be present in air include:

i) Solid particles eg. pollen, dust and smoke
ii) Liquid particles eg. mist and fog
iii) Gases and vapours eg. cooking odours
iv) Microorganisms

The wide range of contaminants presents a problem with air filtration. Smoke particles are in the order of 0.2 microns in size while dust particles may exceed 100 microns (Croome-Gale D.J., Roberts B.M., 1975).

The types of filters in common use are dry filters, wet filters and electrostatic filters. Air washers, mentioned previously, also play a role in filtration, but are relatively inefficient. Ultraviolet filters for the removal

of infectious particles have been used in certain situations.

Dry filters are usually disposable and rely on a fabric or paper-type fibrous material to remove dust particles from the air stream. They may be of either roller-type or panel-type (ACOA Report, 1984). Wet filters are composed of oil coated metal turnings held between wire meshes. They are washable and reusable (ACOA Report, 1984). Electrostatic filters are expensive, but efficient. They will remove material as small as 0.25 microns by ionizing all particles, then collecting the particles on oppositely charged plates (Harris N.C., Conde D.F., 1974).

Ultraviolet (UV) filters consist of mercury vapour discharge tubes transmitting at a wavelength of 253.7nm. UV has a broad range of activity against airborne bacteria and viruses, but is only useful as a surface disinfectant because its penetration properties are poor. Excessive exposure to UV light can produce erythema and conjunctivitis, however, filters situated at ceiling-level can remove infectious particles without harming the occupants (Riley R.L., 1974).

Table II

Comparison of filter efficiency (ACOA Report, 1984)

Type of filter	Minimum particle size (microns)	Blackness efficiency*	Advantages	Disadvantages
Wet filter	5	12%	Low initial cost	Low efficiency with atmospheric dust
Dry filter - rolling	5	20%	Continually selfcleaning	High pressure drop
- low efficiency	5	16%	Low initial cost	Low efficiency on small particles
- high efficiency	3-5	36%-40%		
Electrostatic filter	0.01	95%-97%	High efficiency on both large and small particles. Air pressure and power requirements low	Initial expense, requirement for safeguard against high voltage
HEPA filter**	0.01	99%	Highest efficiency	High pressure drop expensive, costly periodic replacement

* Blackness efficiency test - samples of air are passed through filter paper. The dirtiness of the filter is measured by light transmission. This is the most reliable measure of filter effectiveness.

** High Efficiency Particulate Air Filter

SUMMARY

The main purpose of this chapter has been to describe the basic components of airconditioning systems and explain their function. Future chapters will discuss aspects of microbial contamination of these components and how disease may result. It is felt that with an understanding of both airconditioning and microbiology such diseases may, to some extent, be prevented.

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CHAPTER 2

DISPERSAL OF MICROORGANISMS IN AN AIRCONDITIONING SYSTEM

Conditions favourable to the growth of micro-organisms are sometimes found in mechanical ventilation and airconditioning systems. In fulfilling their primary function, that of air distribution, these systems provide efficient means for the transmission of any contaminants that may be present (Ager B.P., Tickner J.A., 1983).

There are two major types of respiratory diseases associated with airconditioning and ventilation systems, these are:

1. Allergy. A hypersensitivity to inhaled matter of microbial origin, eg. hypersensitivity pneumonitis.

2. Infection. An invasive growth of microorganisms in the respiratory system, eg. Legionnaires' Disease (Ager B.P., Tickner J.A., 1983).

In both cases, the causative agents are microorganisms which, having contaminated the airconditioning system, may multiply in water used for humidification or cooling and be distributed by the air-handling plant into occupied spaces. The common factor in all cases of allergy or infection is contaminated water. Wherever water storing apparatus exists there also exists the potential for causal organisms to grow and be transferred to the building atmosphere (Ager B.P., Tickner J.A., 1983).

Hygienic hazards in airconditioning systems

Growth of microorganisms may occur wherever air comes into contact with water (Gunderman K.O., 1980). Apparatus containing water, such as water-wash units, spray humidifiers, drop separators and cooling towers, provide the most common source of microorganisms in airconditioning systems. Microorganisms may be found in large numbers. Concentrations of 10^6 organisms/ml have been recorded (Gunderman K.O., 1980).

Cooling towers are particularly susceptible to contamination for a number of reasons:

1. They consist of a large body of water.
2. They are open to the environment.
3. Chemical control of microbial growth must be performed carefully in order to achieve concentrations sufficient to discourage growth but not affect occupants through aerosol spread.
4. Temperatures of $30^{\circ}C$ may be achieved in summer months which is the optimum temperature for the growth of some organism including Legionella (Thorpe T.C., Miller R.D., 1980).

There have been many reports of cooling towers acting as a source of Legionella in outbreaks of Legionnaires' Disease (Dondero J.T., Rendtorff R.C., Mallison G.F., 1980; Christopher P., 1984; Band J.D., La Venture M., Davis J.P., 1981).

In Australia, most airconditioning systems do not have a humidifier as it is considered that the air is sufficiently moist (ACOA Report, 1984), however in industry (particularly textiles, paper and printing works), there is a need to control the relative humidity (Ager B.P., Tickner J.A., 1983). Humidifying systems in which water is used as a reservoir, particularly those with recirculation, may provide a suitable environment for a food chain of microorganisms. The range of organisms that develop, will depend on the nutrient content of the water, its pH and temperature. Once an initial colony is established, its metabolic products may provide nutrients for the subsequent appearance of other species. As conditions alter with time, a succession of organisms or a growth chain may result. Such a growth chain may consist of bacteria, fungi, algae, amoebae and nematodes (Ager B.P., Tickner J.A., 1983; ACOA Report, 1984).

A humidifier water tank colonized by a growth chain of microorganisms is likely to form a surface slime on the sides or a crust layer. This material may contain a range of viable and dead organisms along with cell wall components and spores (Ager B.P., Tickner J.A., 1983). The humidifier is a favourable site for allergenic material that may be distributed by aerosolization throughout a building (ACOA Report. 1984).

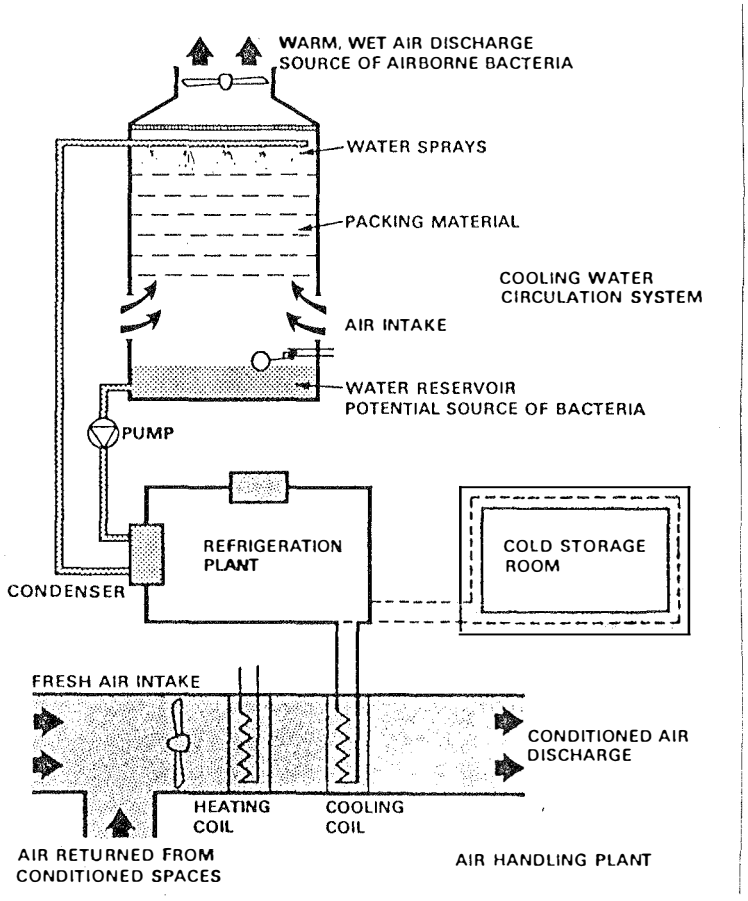
Contaminated ducts constitute the major non-water containing source for airconditioning-related diseases. Ducts are either contaminated during construction and not adequately cleaned before use or alternatively, creeping air movements may introduce organic material into a shutdown system (Gunderman K.O., 1980). The conditions found within ducts such as constant temperature, humidity and moisture plus nutrients in the form of dirt provides a situation in which microorganisms may multiply rapidly (ACOA Report, 1984).

Spread of microorganisms by air currents

Microorganisms contaminating an airconditioning system may be spread throughout the building on air currents. They may be spread horizontally through doorways and along corridors, vertically by means of elevator shafts and stair wells, and in both directions via the ducting system (17th Symposium, 1967). Outside the building, organisms arising from contaminated cooling towers can be sucked into the air inlet to be inhaled by the building's occupants (Dondero T.J., Rendtorff R.C., Mallison G.F., 1980; Ager B.P., Tickner J.A., 1983).

Figure 1

Cooling system for a large building, showing the potential source and discharge of airborne bacteria (shaded area) (Ager B.P., Tickner J.A., 1983).



Airflow in rooms varies widely according to the design and manner of operation of the heating and ventilation systems, the size and shape of the rooms, its furnishings and the activities of occupants. Changes in the weather influence the air movement particularly where the thermal insulation of the building is poor

and where control of natural ventilation is lacking. Some variations occur by chance, others are achieved through design (17th Symposium, 1967). Air currents are usually caused by ventilation, heating or cooling. Initially, the air current is determined by the shape of the air inlets. Air displacement occurs as a result of convection currents through energy transfer. These may be caused by fixed heat sources, such as radiators which produce strong influences on the air current, or less well defined effects, such as people moving within the building causing local turbulence (17th Symposium, 1967).

Approximately 30% of heat generated by a person is lost by convection at a rate of about 100kJoules per hour. Although a weak heat source, the resulting convection stream is surprisingly strong with speeds of up to 20 metres/min being recorded (Croome-Gale D.J., Roberts B.M., 1975). Other disturbances are caused by opening and closing doors and motion within the room. Walking at 3kph creates disturbances that move particles up to 60 metres/min. Such disturbances are of short duration, and dissipate after approximately 15 seconds (Croome-Gale D.J., Roberts B.M., 1975).

Ventilation involves the movement of air into and out of the ventilated space and distribution of the air during its passage through the space. Openings are required to provide access for flow, and force is needed

to overcome the resistance of motion due to friction at the openings and in the space itself. Mechanical ventilation maintains airflow through fan power.

A number of techniques are available to determine the direction of airflow. Most commonly used is a smoke producing device that enables air currents to be "seen". Tracer particles can be produced by heating tablets of metaldehyde. Lightweight crystal particles resembling dandelion seeds are formed which can remain suspended in the air for fifteen minutes. The particles are illuminated by collimated light sources (17th Symposium, 1967). Another method is to spray potassium iodide particles which can be developed, following dispersal, with palladium chloride producing visible dark brown spots (Foord N., Lidwell O.M., 1972). Viable bacteria, including Serratia marcescens and Bacillus subtilis have been used to demonstrate airflow (Silver I.H., 1970). The organisms are aerosolized and retrieved by gravity settling plates or an air sampling device. The danger of viable organisms causing infection precludes this technique from being employed in occupied buildings. Another method involves the release of halocarbons. Air samples are collected and the halocarbons detected using gas liquid chromatography (17th Symposium, 1967).

Dispersal of microorganisms on particulate matter

The ability of a particle to remain airborne, its ability to pass through filters, the site at which it may be deposited and the rate at which it will be removed by sedimentation are all dependent on the size and density of the particle (Noble W.C., Lidwell O.M., Kingston D., 1963).

Airborne transmission of disease occurs through three vehicles: dustborne microorganisms, organisms carried in droplets and organisms that constitute droplet nuclei (Walter C.W., 1966). Bacteria appear to travel exclusively in this fashion although fungi may be present in air as single spores (Noble W.C., Lidwell O.M., Kingston D., 1963).

Dustborne bacteria are derived from fragmentation of dried excrement, excretions or discharges, or from desquamated epithelial cells (Walter C.W., 1966). In studies of hospital dust, epithelia were present in abundance (Davies R.R., Noble W.C., 1962). Epithelia provide a 'raft' for bacteria that is within the size range 4-20um required for airborne spread (Noble W.C., Lidwell O.M., Kingston D., 1963). They are shed from the body at such a rate that a complete body layer is discarded every two to three days (Davies R.R. Noble W.C., 1962).

Microorganisms present in droplets and as droplet nuclei may arise from atomizing-spray type humidifiers and cooling towers (Ager B.P., Tickner J.A., 1983). Cooling towers discharge large volumes of moisture-laden air to the surrounding atmosphere. Microorganisms may be present in the moisture or, following dehydration, as droplet nuclei. Droplet nuclei remain airborne indefinitely and may penetrate into the alveoli of the lungs where they may initiate pulmonary infection (Walter C.W., 1966).

Summary

Microorganisms may be present in any water-containing apparatus within the airconditioning system and also in ducts. These sites should represent the focus of attention for prevention of contamination and also the source of specimens for investigation into air-conditioning related diseases.

Microorganisms present in ducts can be spread on dust particles and epithelia. Contaminated spray humidifiers, cooling towers and other water-containing devices are able to spread organisms by water droplets or as droplet nuclei. Such particles have a potential for infectivity either through allergic reaction or tissue invasion.

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CHAPTER 3

COLLECTION AND HANDLING OF SPECIMENS

The examination of airconditioning systems for evidence of microbiological contamination may be regarded as either a routine quality control exercise or a specific investigation to determine the source of an infection. In order to restrict the field of investigation, it is worthwhile considering the aetiologic agents involved in the two major diseases related to airconditioning systems, Legionnaires' disease and humidifier fever.

Legionnaires' disease: includes Legionella, Tatlockia and Fluoribacter species and probably other Legionella-like organisms as yet unclassified. (Edelstein P.H., 1985).

Hypersensitivity pneumonitis agents: reaction has been shown to a wide range of microorganisms including fungi, bacteria and protozoa. Inorganic material such as dust and smoke has also been implicated. Whilst it is unnecessary to isolate and identify the actual component producing the allergy (and in fact it may prove to be too dificult), it is important to provide evidence that air-conditioning related debris is producing the disease (Anonymous, Lancet, 1980).

Specific methods for processing specimens from cases of Legionnaires' disease and humidifier fever will be covered in chapters 5 and 6 respectively.

Collection and processing of specimens from air-conditioning systems

1. Water

Organisms grow whenever air comes in contact with water. Cooling towers, humidifying boxes, water wash units, drop separators and source water may become contaminated (Gunderman K.O., 1980).

Large volumes of water, 2 - 10 litres, should be removed aseptically from these sites. There is no set procedure for collection, however, standard techniques for collecting potable water can be modified to suit. The specimen should be representative of the body of water. A sterile container is immersed in the water, then the lid removed and the container filled without rinsing. A space is left for mixing purposes. A small volume of sodium thiosulphate, 0.1ml 3% solution per litre of water, may be added to counteract the effect of chlorine in the sample (Anonymous, 1961). The use of long, sterile (eg. surgical) gloves will serve to maintain aseptic conditions and protect the person collecting the sample.

Unless the water is visibly turbid, large volumes will need to be concentrated to ensure that all contaminants will be isolated. The two concentration methods available are filtration and centrifugation.

Filtration

This procedure is generally performed using a millipore filter although other filters are available. To ensure all bacteria are trapped, a pore size of less than 0.75 microns is necessary. 0.45 and 0.20 micron filters are most commonly used. The filter can either be placed directly on to an agar plate or rinsed in sterile saline or Ringer's solution to remove all organisms, then inoculated on to the required media (Collee J.G., Duguid J.P., Scott A.C., 1975).

Millipore filters have been used successfully by placing them directly into shower roses to collect organisms present in source water (Anonymous, 1981).

Several problems may be encountered with the filtration technique. A filter placed directly on to an agar plate can result in a heavy concentration of organisms which may overgrow the desired one. Washing the filter first, then performing dilutions may overcome this, but is not guaranteed to free all organisms. Turbid water may clog the filter with debris, decreasing its efficiency.

Centrifugation

Large volumes of water can be processed by this technique. The resulting deposit allows for easy manipulation either by dilution or direct inoculation. A number of different media can be inoculated from the same sample.

The trauma involved during centrifugation may damage bacterial cells, thus reducing viability. The use of a refrigerated centrifuge may improve recovery but some cells will still be damaged (Collee J.G., Duguid J.P., Scott A.C., 1975).

Water is considered the most useful specimen that can be collected from airconditioning systems. Both Legionnaires' disease bacilli and organisms implicated in hypersensitivity pneumonitis are isolated from water-containing apparatus.

2. Filters

Filters are found in various sites throughout the airconditioning system, at the inlet, the plenum and sometimes at the inlet to a room or specific region (Croome-Gale D.J., Roberts B.M., 1975). They may be cultured to determine the range of organisms present and to provide an indication as to the region in which contamination may be a problem. Filters can be processed by cutting them into appropriate sizes, adding fluid, such as sterile saline or Ringer's solution, then vortexing in order to remove trapped particles. The fluid can be further diluted or concentrated, then inoculated on to the required media.

3. Dust

Debris left in the ducts following their installation, or acquired through creeping air movement and air intake

may account for dust in the ductwork (Gunderman K.O., 1980). This may harbour organisms particularly fungal spores like Aspergillus species to which some people are allergic. Other organisms, some as yet unidentified, and the dust itself can produce an allergic response.

Swabs or scrapings of the debris may be collected for culture, double gel diffusion against the patients' serum, or challenge studies (Anonymous, 1980).

Air Sampling

Air that has been processed through the heating, humidifying and filtering procedures represents the final product of the airconditioning system. Air is the medium which transports organisms from the site of contamination to the susceptible host. It is apparent that the purity of processed air is of considerable importance to the well-being of the building's occupants.

Air sampling devices can be operated within the air-conditioning ductwork and at outlet sites to determine the microbiological status of the air and also if specific organisms of interest are present. If a regular check is made and records kept, it will become obvious if the air quality is decreasing (Anonymous, PBI). Other parts of the building can be tested, however, it must be noted that organisms cultured from occupied regions will mostly have originated from the occupants.

The question of air purity is of such fundamental importance that a variety of equipment has been developed for the sampling of microorganisms in air. A number of difficulties have had to be overcome, including the wide range of organisms that can be encountered, from viral units of less than 0.1um to fungal spores of 50um - 100um. Small viable particles may be attached to much larger "rafts" of other materials such as epithelial cells, fibres and dust particles. This diversity means that sampling equipment generally has a narrow scope and several machines are required if a full analysis is to be performed (May K.R., 1966).

The extraction of airborne particles may be considered as two distinct steps. Firstly, the process of drawing the stream of air into the sampling apparatus with as little change of concentration as possible and secondly, the process of depositing airborne particles on or in a suitable medium for identification and counting (Treskunov A.A., 1971).

Air Samples

i) Sedimentation

This technique requires a petri dish to be exposed to ambient room air for a prescribed period of time. Routine studies generally use horse-blood agar plates, however, any nutrient medium may be employed. Aerosols of bacteria can be highly dilute necessitating the sampling of large volumes of air. Sedimentation plates

should, therefore, be exposed for long periods, usually one to eight hours (May K.R., 1967). Problems will occur with dessication which may be overcome by pouring thicker plates or by covering the agar with a monolayer of oxyethylene docosanol (May K.R., 1969).

Sedimentation plates are probably the most widely used air sampling device because of their simple and inexpensive application. This method is not recommended for quantitative evaluations because it mainly collects particles that have sufficient mass to be deposited by gravity or impacted by air turbulence onto the collecting surface. Airborne particles that are too light to settle out quickly may not be collected (Sayer W.J., MacKnight N.M., Wilson H.W., 1972).

In a study on the efficiency of the sedimentation plate versus the Anderson' Sampler, 37 of the 53 paired tests revealed organisms isolated from the Anderson Sampler that were not detected by the sedimentation plate (Sayer W.J., MacKnight N.M., Wilson H.W., 1972).

ii) Impaction on solid surfaces

A vacuum source is required to draw air through the sampler and deposit organisms onto a solid surface. In the Anderson Sampler, air enters through a sieve while in the Reynier and Fort Detrick Samplers, air is drawn through a slit. The air intake is measured so that a quantitative result can be achieved. This is expressed on the number of organisms per cubic metre of sampled air (May K.R., 1967).

The Anderson Sampler is the most commonly used air sampler. The original instrument consists of six stages (Anderson A.A., 1958). Later models have eight stages, whilst a disposable instrument is available with only two stages (Curtis S.E., Balsbaugh R.K., Drummond J.G., 1978). Each stage contains a plate perforated with 400 holes and immediately beneath an agar plate is placed. Air is drawn through the instrument at a specified rate and a jet of air from each of the holes plays on the agar surface. The hole size is smaller for each succeeding stage. A particle will be drawn down each stage until its mass and the air velocity combine to impact it onto the agar (Anderson A.A., 1958).

Figure 1

Cross-sectional schematic diagram of an Anderson Sampler (Sayer W.J., MacKnight N.M., Wilson H.W., 1972)

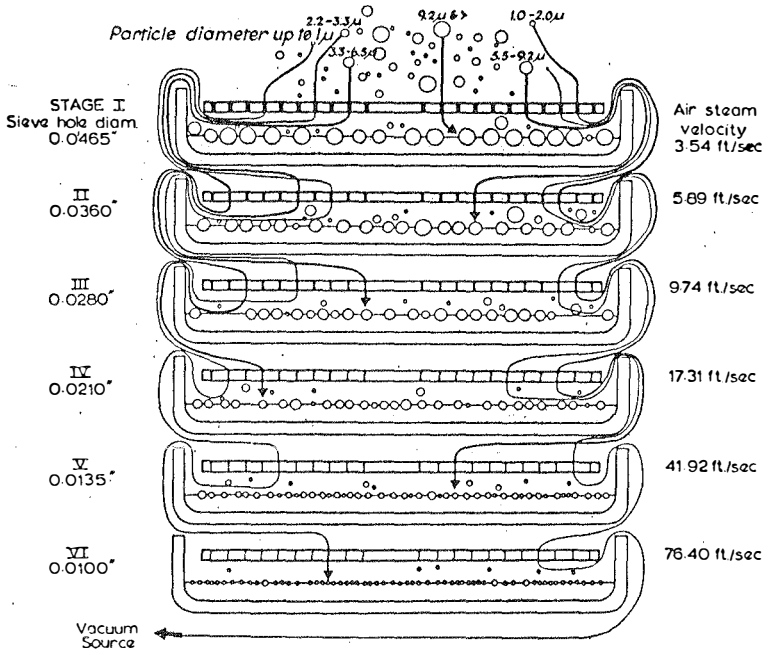

FIG. 1. Cross-sectional schematic diagram of an Andersen or sequential impaction cascade sieve volumetric air sampler. Note the decreasing sieve-hole size with increasing airstream velocity from top to bottom. Idealized particle spheres are greatly magnified to illustrate the collection of particles and the classification by sizes on the media surfaces of the six stages.

The six stage instrument permits the retention of particles from 9.2um to less than 1um. Few particles larger than 4-5um reach the lungs as nasal efficiency for screening particles greater than 5um is virtually 100%, and decreases to 0% for particles of 1um. Consequently, for an instrument to assess the potential of aerosols to produce disease it must be able to determine both the number and size of airborne particles (Sayer W.J., MacKnight N.M., Wilson H.W., 1972).

Modifications of the Anderson Sampler can be used to isolate viruses by increasing the air intake capacity to 10-20,000 litres/min and using petri dishes containing semi fluid media designed to trap virus particles. The catch is processed through cell-line culture or through immunological techniques.

iii) Impingement in liquid

Instruments that project the particle into liquid have an advantage in that individual component cells are broken up. The liquid can then be further diluted, which is important if the concentration of organisms is high. It is useful in that a number of different media can be inoculated from a single specimen and also that a total viable count can be performed.

The impinger is simple, cheap and easily sterilized and the addition of a pre-impinger allows for an approximate parallel to the respiratory system. The most commonly used instrument is the All-Glass-Impinger (AGI-30)

which operates at a maximum airflow of 12.5litres/min. This rate is too low for viral isolation, however, large volume liquid scrubbers operating at over 1000 litres/min are suitable. The impingement fluid can be subcultured on to cell lines and also serological and RIA techniques can be performed directly on the fluid (May K.R., 1966).

iv) Centrifugation

Airborne particles are deposited on to a solid medium by centrifugal force. The incoming airstream is bent and particles, by virtue of their mass, are incapable of making such a sharp turn and are impacted on to the medium. An example of this technique is the Well's Centrifuge (Loughhead H.O., Moffett J.A., 1971).

v) Filtration

A measured quantity of air is filtered through a membrane which can then be incubated on solid media (Loughhead H.O., Moffett J.A., 1971).

vi) Electrostatic Precipitation

Agar plates are exposed on both a positive and negative electrode. A known volume of air is drawn over the plates with particles being attracted by virtue of their electric charge. This technique is highly efficient but complex and expensive. A modification has been developed for viral isolation using cylinders coated with liquid (Loughhead K.O., Moffett J.A., 1971).

vii) Thermal Precipitation

Airborne particles are repelled by hot surfaces and deposited on cold ones. This property has

been employed to recover airborne particles through thermal gradients, but is slow and complex (Loughhead H.O., Moffett J.A., 1971).

viii) Airborne Particle Counter

The Airborne Particle Counter is an expensive instrument but has the advantage over conventional counters in being able to provide immediate results. The size of particles in the air is determined and from this a correlation can then be made with expected bacterial contamination (Loughhead H.O., Moffett J.A., 1971).

Human and Animal Specimens

The first indication of a contaminated airconditioning system is usually a grouping of cases of respiratory disease in which all those involved have had association with a particular building or area. The obvious choice of specimens are those arising from the respiratory tract, commencing with those most easily obtained, then biopsy and possibly necropsy material. Thus, the following specimens may be obtained: sputum, transtracheal aspirates, pleural aspirate, bronchial washings, lung tissue and blood for both culture and serological analysis (Feeley J.C., Gorman G.W., 1980).

It is essential that suitable specimens be collected as soon as possible so that there is no interference from antibiotics. An acute specimen of sera is vital, not only to determine single raised antibody titres, but also as a

reference point so that if a diagnosis is not made, then acute and convalescent sera may be stored for retrospective analysis. Similarly, any histological specimens are also stored for further study (Feeley J.C., Gorman G.W., 1980).

Patients suspected of having hypersensitivity pneumonitis can be tested to determine the source, if not the actual allergens, by gel diffusion studies or challenge with suspected material (Anonymous, 1980).

Animals have played a role in investigative studies of airconditioning systems. In the case of a Legionnaires' disease outbreak in a British hospital, the airconditioning ducts were connected to heavy duty plastic bags containing athymic nude mice and guinea pigs. Following exposure, the organs were processed for the presence of microorganisms. Pre and post exposure sera were collected for serological analysis (Fischer-Hoch S.P., Tobin J.O'H., Nelson A.M., 1980).

Animals, particularly guinea pigs, may be used to filter out pathogenic organisms from specimens contaminated with other organisms such as may occur in cooling tower water and respiratory tract secretions. Generally, the animal is able to withstand intraperitoneal inoculation with organisms from the environment and those constituting normal oropharyngeal flora, but will be affected by more pathogenic organisms such as Legionella species (Feeley J.C., Gorman G.W., 1980).

Retrospective Studies

Once a case or cases of disease attributable to the airconditioning system has been discovered, further investigation often reveals other cases. In some instances stored sera or histological blocks can be tested to provide conclusive evidence that the patient had a certain disease. Record searching may also reveal diagnosed cases that belong to an epidemic situation.

Environmental Specimens

The focal source of an infection may be traced to a contaminated cooling tower or other appliance, however, the primary source may be found to be a nearby body of water or recent excavation. Water and soil samples from surrounding areas may be useful (Fraser D.W., 1980).

Birds and animals may be responsible for transmission of organisms from the environment to the airconditioning system. Drift of particles on prevailing wind currents is another possibility (Fraser D.W., 1980).

Handling the Catch

All airborne organisms should be regarded as being potentially damaged. The significance of damaged organisms is subject to debate. There is evidence to suggest that damaged organisms which can still form colonies on suitable media may be relatively incapable of causing disease.

It has been proposed that only organisms capable of growing under adverse selective conditions are of medical importance (Kingston D., 1971).

Airborne organisms are usually dry and it is to be expected that the cell membrane will not function properly during rehydration. Dried organisms should be rehydrated in solutions which do not upset the internal environment of the cell. Media with a high sugar content has been shown to improve the survival of bacteria (Kingston D., 1971).

Some media will form peroxides if subjected to ultraviolet light from either indirect or direct sunlight. Other media may spontaneously form peroxides. Damaged organisms are more sensitive to peroxides than undamaged ones, thus media containing sodium pyruvate, blood or other peroxide-destroying agents should be used in the recovery medium (Kingston D., 1971).

A major problem in the isolation of microorganisms under field conditions is that the desired organism is often present in low concentrations whilst other, unwanted organisms greatly outnumber it. Selective techniques are necessary to isolate specific organisms. During the rehydration of microorganisms, the interior of the cell is unusually accessible to selective agents so that any selective procedure must be rigorously tested before use (Kingston D., 1971).

Damaged organisms on culturing may produce colonies that are atypical and slow in development (Kingston D., 1971).

Selective Agents

i) Nutritional

It is possible to include in media nutritional additives that allow the required organisms to grow but which do not support the growth of unwanted organisms. Generally, however, nutritionally exacting organisms are being examined from a background of organisms that are less fastidious. An example of the use of a nutritional selective agent is the use of paraffin incorporated in agar for the isolation of Nocardia species (Kingston D., 1971).

ii) Atmospheric Requirements

Obligate aerobes or anaerobes may be selectively removed by incubating culture plates in either an anaerobic or aerobic atmosphere, respectively (Kingston D., 1971).

iii) Temperature

Little work has been performed on the use of temperature as a selective agent. Thermophilic Actinomycetes can withstand temperatures of 56°C, whilst 41°C is a useful temperature for the selection of Staphylococcus aureus in air sampling (Kingston D., 1971).

iv) Antibiotics

A wide range of antibiotics has been used in selective media. Minimum inhibitory concentrations (MIC's) are readily available and assist with the determination of the antibiotic concentration required.

Several antibiotics are in general use. They are: Polymyxin B at 1-10mg/l to suppress Gram negative bacteria, a combination of penicillin and streptomycin each at 100mg/l to suppress bacteria when isolating fungi, Actidione is used to suppress moulds where prolonged incubation is necessary (Kingston D., 1971).

v) Other Selective Agents

MacConkey agar containing bile salts has been used successfully to isolate members of the Enterobacteriaceae. Bacillus anthracis may be isolated using a medium containing propamide isothionate at 100mg/l (Kingston D., 1971).

Alternatives to Selective Media

i) Indicator Medium

Readily identifiable colonies may eliminate the need for reducing the background flora. Examples of this technique are: media containing phenolphthalein phosphate for Staphylococcus aureus and Nagler's media for identifying Clostridium perfringens (Kingston D., 1971).

ii) Mechanical Separation

Differential centrifugation can separate bacteria from viruses, and fungal spores from bacteria (Kingston D. 1971).

iii) Animal Inoculation

Various organisms, including Legionella, have been isolated by their ability to give rise to infections

when inoculated into susceptible animals. The animals are thus acting as a growth selective medium. Parenteral inoculation is generally more sensitive than inoculation through inhalation (Feeley J.C., Gorman G.W., 1980).

iv) Subsequent Transfer

Organisms can be grown initially on non-selective media, then transferred once grown and prior to overgrowth from other organisms, to more selective conditions. The selective agent acts on a larger number of organisms in a healthier condition thus improving recovery chances (Kingston D., 1971).

Identification of Microorganisms

Bacteria

Bacteria may be presumptively identified by their cultural and Gram morphology and also their atmospheric and growth requirements. Direct fluorescent techniques, specific stains, latex agglutination and serological tests provide rapid and usually accurate identification. Numerous biochemical tests, some simple and some requiring sophisticated instrumentation may be required to speciate an organism (Baker F.J., Breach M.R., 1967).

Fungi

Careful examination of the macroscopic and microscopic details are the most important procedures in the identification of fungi. Microscopic features such as the presence and appearance of microaleuriospores, macroaleuriospores,

sporangia, hyphae, sterigmata, columella, rhizoids and ascocarps are often characteristic. It is occasionally necessary to perform nutritional or biochemical tests in order to speciate the fungi. The following such tests are employed: thiamine, inositol, xanthine and tyrosine utilization, urease, proteolysis and starch hydrolysis. Yeasts are identified by sugar assimilation tests, macroscopic appearance and microscopic appearance on cut-streak cultures (Al-Doory Y., 1980).

Viruses

Serological tests are available such as the Direct and Indirect Fluorescent Antibody Tests, Radio Immuno Assay and Enzyme Linked Immuno-Sorbent Assay. Viruses may be isolated by inoculating material into culture systems such as tissue culture, embryonated egg, suckling mice and membrane pieces. Identification can then be achieved by examination of the cytopathic effects and neutralization reactions (Timbury M.C., 1971). It should be noted that viruses are obligate intracellular parasites and as such are incapable of multiplying in non-living systems

Amoebae

Amoebae are identified by their method of locomotion, size and stained morphology (Ash L.R., Orihel T.C., 1980).

Other Airborne Particles

A vast array of particles may be isolated from the air. Perhaps the most important of the organic particles are pollen grains which can be recognized by their uniformity of structure. Desquamated fragments of human skin, lichen-thallus, plant hairs, textile fibres, algae and other material may be present (May K.R., 1967).

Interpretation of Results

The capacity to grow, isolate and identify microorganisms also requires the ability to determine the significance of such results. One method is to chart culture results from the area under investigation maintaining a uniformity of test procedures. A deviation from normal readings can then be detected (Anonymous PBI).

An alternative technique is to examine the specimens for marker organisms which indicate the likelihood of contamination. Present bacteriological practice is based on showing the possibility of pollution existing rather than showing the actual disease producing organisms are present (Anonymous, 1961).

SUMMARY

The most valuable specimen that can be obtained from airconditioning systems in the event of an air-conditioning related disease or for quality control purposes, is water. Other specimens that are occasionally sought are filters and dust samples. Water-containing apparatus should be inspected regularly and specimens collected when appropriate. Concentration procedures such as filtration and centrifugation are usually necessary to increase the microbial yield.

Air sampling can be employed to investigate air movement in cases of airconditioning related diseases so that the original source of infection can be determined. This technique is also used in quality control programmes to obtain an ambient microbial air concentration. Variations from this level should be investigated.

Serum specimens are essential to the diagnosis of both Legionnaires' disease and hypersensitivity pneumonitis and should be collected as early in the disease as possible. The methods for processing serum specimens will be discussed in the relevant chapters.

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CHAPTER 4

PULMONARY DEPOSITION AND RETENTION OF INHALED PARTICLES

Water-containing apparatus within the airconditioning system is able to produce droplets and droplet nuclei that contain microorganisms. Dust and epithelial cells within the ducts are also vehicles for the transmission of organisms.

This chapter covers the anatomy and physiology of the respiratory tract and the effects that airborne particles have on it. This section will also include the nature of particles required to initiate infection.

The Respiratory System

Development

During intrauterine development, the lungs pass through a glandular, canalicular and alveolar phase.

The first four months comprises the glandular stage whereby the lung bud grows rapidly. Newly formed bronchi divide further in the canalicular phase, while the airways terminate into relatively wide saccules from which the alveoli develop. The alveolar period begins in the final eight to ten weeks of pregnancy, although the alveoli are shallow and small. Most alveoli are formed by segmentation of existing alveolar units (Bouhuys A., 1974).

Surfactants, which are composed of surface active phospholipids, mostly phosphatidylcholine (lecithin), are synthesized by alveolar epithelial cells (type II pneumocytes) and secreted to form an alveolar surface lining. This decreases surface tension at the air-liquid interface and maintains the stability of the alveoli (Robbins S.L., Cotran R.S., Kumar V., 1984).

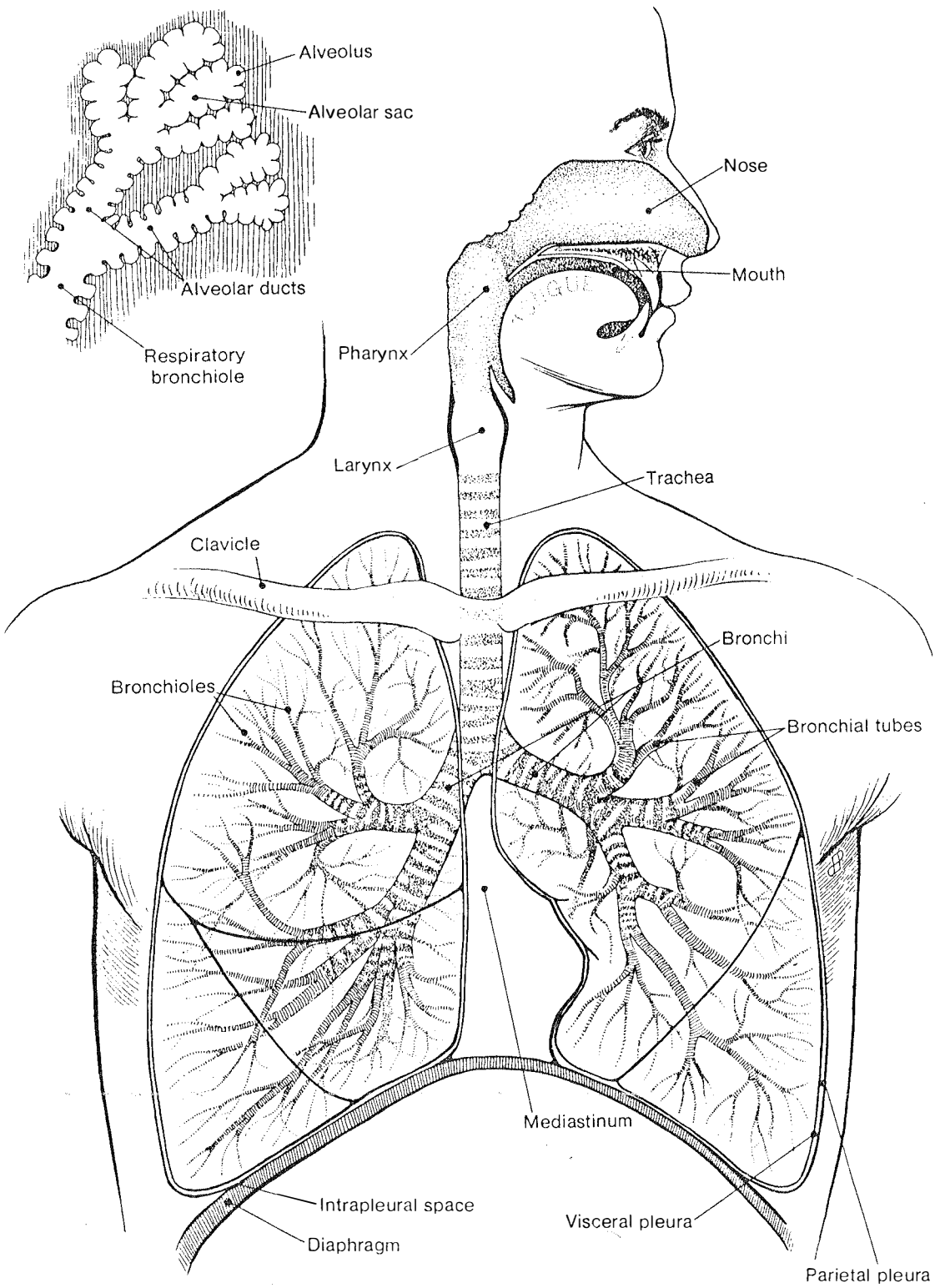
As children grow, their chest cages and lungs become larger. Total lung capacity, vital capacity and maximum expiratory flow rates increase, while airflow resistance in the bronchi decreases (Steen E.B., Montagu A., 1964).

Anatomy

The respiratory system is usefully described in three sections: the nasopharynx; the respiratory airways, beginning at the trachea and ending with the terminal bronchioles; and the lungs within which oxygen and carbon dioxide is exchanged between respired air and blood (Steen E.B., Montagu A., 1964).

Figure 1

The Respiratory System (Watson J.E., 1972)



The Nasopharynx

The nasal cavity is divided into passages by a septum, then further, into fissures and channels which cause the oncoming air to be exposed to a maximal surface area. This serves to warm and moisten the air, and by sudden changes in direction of airflow, to cause impingement and entrapment of some of the larger suspended particles (Hatch T.F., Gross P., 1964).

The respiratory portion of the nasal passages is covered by a mucous membrane consisting of ciliated columnar epithelial cells interspersed with mucin-secreting goblet cells. Because of ciliary action, the sticky surface is constantly on the move promoting the clearance of foreign matter from the pharynx (Steen E.B., Montagu A., 1964).

The pharynx begins below the level of the soft palate and terminates at the epiglottis. It is a muscular tube that is shared with the digestive system. The epithelial lining is predominantly stratified squamous or mucin-secreting, columnar, ciliated epithelia (Spence A.P., 1982).

Respiratory Airways

Commencing with the trachea, the airways are composed of branching tubes progressively reducing in size and cross-sectional area. This decreases the velocity of inflowing air, whilst the branching effect serves to distribute the air as uniformly as possible (Steen E.B., Montagu A., 1964).

The trachea is the tube continuing from the larynx to the bronchi. It is lined with a mucous membrane of pseudostratified ciliated columnar epithelium that contains numerous goblet cells. The cilia beat upwards tending to carry foreign particles away from the lungs and up to the pharynx.

The trachea divides into the left and right primary bronchi and then into secondary bronchi. These in turn branch into tertiary bronchi, bronchioles and terminal bronchioles (Spence A.P., 1982).

The Lungs

The right lungs comprises three lobes whilst the left has two. Each lung is enclosed in a double-walled sac called the pleura between which the pleural cavity is moistened with pleural fluid.

Within the lung, the terminal bronchioles divide into two respiratory bronchioles which further subdivide so that each terminal bronchiole ultimately gives rise to 14 respiratory bronchioles. Each respiratory bronchiole divides into two alveolar ducts, the total number of which has been estimated at between 2.6×10^7 and 4×10^8 (Hatch T.F., Gross P., 1964).

The air in the alveoli is separated from the blood by a very thin respiratory membrane formed by the alveolar epithelium. This is composed of capillary endothelium,

basement membrane, type I and type II pneumocytes (Robbins S.L., Cotran R.S., Kumar V., 1984).

Physical Factors in the Respiratory Deposition of Aerosols

There are three main forces which operate within the respiratory tract to deposit particles from the inhaled air.

i) As the air flows in and out of the lungs, inertial forces cause particles to be impinged upon the nasopharyngeal mucosa and wherever branching of the airways causes a change in the direction of airflow. Effectiveness of this system is dependent upon air velocity and consequently decreases with depth into the respiratory system.

ii) Gravity settlement occurs within the finer airways and air spaces of the lungs in amounts proportional to the duration of time available for settlement. Sedimentation is favoured by depth into the respiratory system because of the reduction in air turbulence.

iii) Particles the size of gas molecules are deposited on the walls in the finest airways, by diffusion, the result of constant bombardment of the particles by vibrating gas molecules (Hatch T.F., Gross P., 1964).

There is an upper limit to the size of particles that can be inhaled due to settling velocity, the atmosphere and other factors. Most large particles are deposited by inertial impaction in the nasopharynx. Sedimentation due

to gravity is important within the bronchial tree, whilst in the peripheral lung units, brownian movement is the main cause of particle deposition (Bouhuys A., 1974).

Deposition of particles depends on their size, shape and density. The dimensions of particles entering the bronchial tree also depends on the breathing pattern and the anatomy of the airways for impaction. During quiet nasal breathing most particles > 10um are probably deposited in the nose. As tidal volume increases,the nose filters out progressively smaller particles due to the increase in inertial forces, so that impaction is more likely to occur (Bouhuys A., 1974).

It has been shown that most particles between 0.3um and 1.0um diameter are deposited in the alveoli and terminal bronchioles. Particles above 1.0um are deposited in the terminal bronchioles only (Beekmans J.M., 1965). Particles > 4.5um are not considered likely to be deposited in the lungs (Curtis S.E., Balsbaugh R.K., Drummond J.G., 1978).

Infectious Particles

Within the airconditioning system, microorganisms can be picked up on aircurrents as a spray or aerosol. The droplets are rapidly dried, leaving "droplet nuclei" approximately 1um-3um in size. The droplet nucleus is an efficient infecting particle and the airhandling sys-

tem provides the avenue for which the particle can reach susceptible lungs (Riley R.L., 1974).

Droplet nuclei have a negligible settling capacity and travel wherever the air goes. Transmission of infection by droplet nuclei is an indoor phenomenon being limited to confined areas where the particles can reach concentrations which constitute a hazard to susceptible people (Riley R.L., 1974).

Small infective particles remain airborne and have the potential to cause infection until they either die, are killed or are vented to the outdoors. In order to establish disease, the microorganisms must retain viability and be deposited deep in the lungs (Hatch T.F., Gross P., 1964). In the case of allergic reactions (eg. hypersensitivity pneumonitis), viability is not required, however, the allergen must be deposited in the alveoli (Robbins S.L., Cotran R.S., Kumer V., 1984). Droplet nuclei are more efficient infecting particles than are droplets or dust as they are readily deposited in the alveoli and fewer particles are required at this site to produce infection. Dustborne bacteria and droplet particles are generally larger than 10um and are deposited in the nasopharyngeal chamber and upper respiratory tract (Hatch T.F., Gross P., 1964).

Pulmonary Defence Mechanisms

Each day the respiratory system is exposed to approximately 10,000 litres of air containing dust, chemicals

and infectious microorganisms. However, the normal lung is sterile and although the amount of material inspired during a lifetime is several hundred grams, only a few grams of mineral dust is found at autopsy. This indicates that inhaled particles are removed by a clearance mechanism (Robbins S.L., Cotran R.S., Kumar., 1984).

In the upper respiratory tract, particles deposited on the non-ciliated epithelium at the front of the airway are removed by sneezing or blowing. Those deposited over mucus-lined ciliated epithelium are carried with the moving blanket of mucus up to the nasopharynx, where they are swallowed (Bouhuys A., 1974).

Tracheobronchial clearance is achieved by mucociliary action. The beating motion of cilia moves a film of mucus continuously from the lungs towards the oropharynx. Particles deposited on the mucous film are swallowed or expectorated (Robbins S.L., Cotran R.S., Kumar V., 1984).

The properties of the mucous blanket are important factors in the clearance mechanism. The components are derived from three sources: the fluid covering the alveolar membrane, the mucus-secreting cells lining the respiratory bronchioles and the goblet cells of the tracheobronchial mucosa (Bouhuys A., 1974).

In experimental studies, the transport of particles placed upon the surface of excised tracheas averaged 15mm per minute. Other studies have shown 50% clearance of 4um iron oxide particles from the upper respiratory tract within 90 minutes, with essentially complete clearance of 1.5um

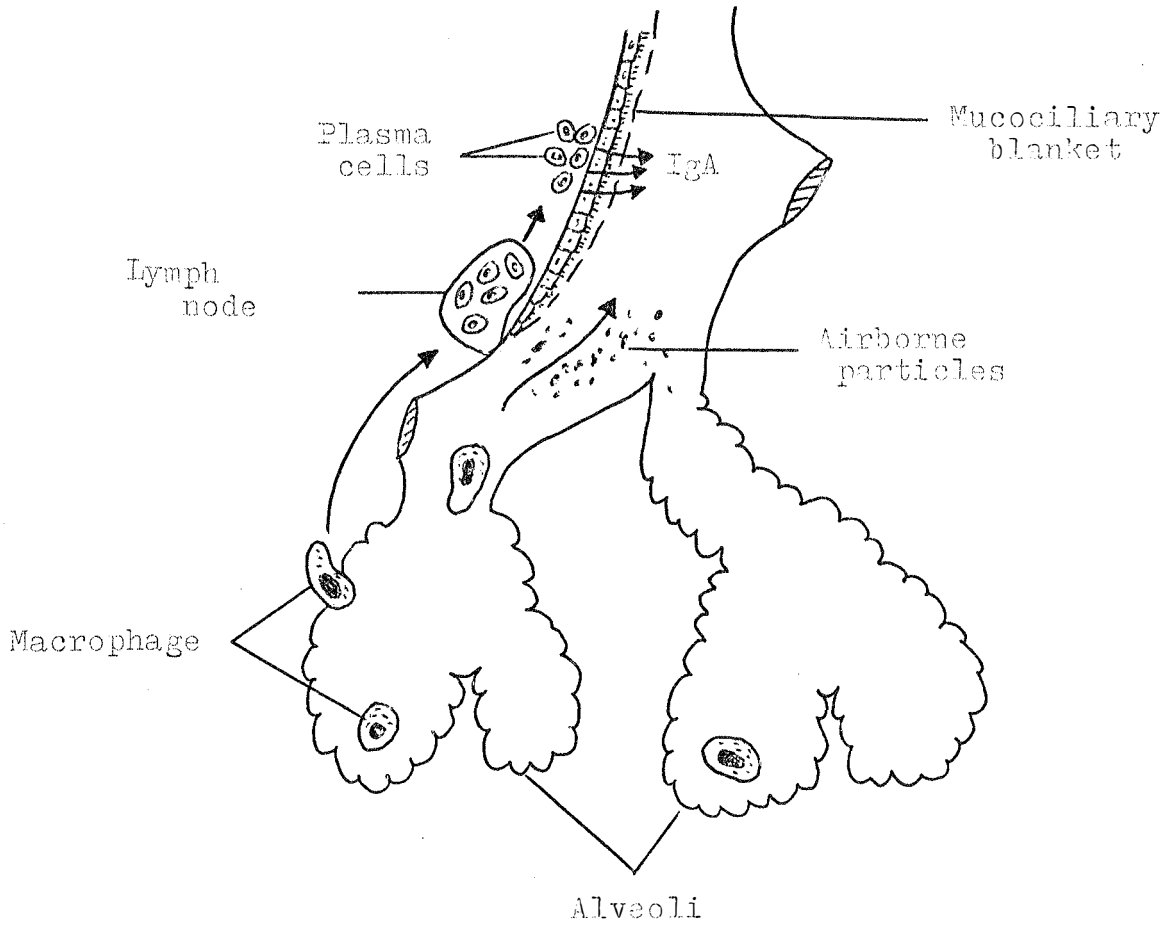
antimony trioxide particles from guinea pigs in six hours (Bouhuys A., 1974).

Bacteria or solid particles deposited in the alveoli are phagocytosed by alveolar macrophages which remove the particles either by digestion or by carrying it to the ciliated bronchioles. The macrophage is transported on the mucous blanket to the oropharynx and then swallowed. If the particle load is heavy, the macrophage may move through the interstitial space and reach the regional lymph nodes from where it can be transported throughout the body via the bloodstream. In the event of a massive exposure to infective particles, an acute inflammatory reaction occurs with polymorphonuclear leucocytes aiding in phagocytic and bactericidal functions. If the particles are antigenic, then immunoglobulins will be produced by plasma cells.

Figure 2

Components of the lung's defences

(Robbins S.L., Cotran R.S., Kumar V., 1984)



In order to avoid an accumulation of particles within the lungs, the rate of removal must exceed the rate of deposition. The healthy lung can achieve this balance with light particle loads. If the particle load deposited in the alveoli is high, or if the clearance mechanisms are impaired, then infection or a sequestering tissue reaction known as pneumoconiosis occurs which may be followed by other pathologic events (Schlueter D.P., 1974).

There are a number of factors that interfere with the clearance mechanism :

i) Loss or suppression of the cough reflex as a result of coma, anaesthesia or neuromuscular disorders.

ii) Injury to the mucociliary apparatus through impairment or destruction of the cilia as a result of cigarette smoking, gas inhalation, viral disease or genetic disturbances (eg. immotile cilia syndrome).

iii) Interference with the phagocytic or bactericidal action of alveolar macrophages due to such factors as alcohol, tobacco, anoxia and oxygen intoxication.

iv) Pulmonary congestion and oedema are probably the most common predisposing factors to terminal bronchopneumonia in patients with congestive cardiac failure.

v) Accumulation of secretions in conditions such as cystic fibrosis and bronchial obstruction (Robbins S.L., Cotran R.S., Kumar V., 1984).

vi) Increasing age results in a decrease in the phagocytic activity of macrophages and in the activity of the cilia of the epithelium lining the respiratory tract (Mason E.B., 1983).

In debilitated patients it is common to find one type of pneumonia predisposing to another. For example, the cause of death in serious influenza epidemics is often bacterial pneumonia. It is likely that the viral pneumonia affects both the clearing mechanisms and the host's immune mechanisms leading to infection with the second agent (Robbins S.L., Cotran R.S., Kumar V., 1984).

The portal of entry for most pneumonias is the respiratory tract, however, haematogenous spread from one focus to another can occur. It may be difficult to distinguish secondary pneumonia from primary pneumonia (Robbins S.L., Cotran R.S., Kumar V., 1984).

Patients hospitalized with chronic diseases frequently acquire pneumonia that results in death. The risk of infection is increased in the hospital situation because of bacteria with acquired resistance to antibiotics, increased opportunities for spread during nursing procedures, invasive techniques such as catheterization allowing a portal of entry (Robbins S.L., Cotran R.S., Kumar V., 1984) and the increased concentration of atmospheric microorganisms that may result from airconditioned buildings (Riley R.L., 1974).

SUMMARY

The respiratory tract consists of a nasopharynx, respiratory airways and lungs. These structures are basically tubes progressively reducing in size and cross-sectional area. The tubes terminate in the lungs as alveoli where gas exchange occurs.

Inhaled particles are precluded from reaching the lungs by impingement on to the mucous surface of the upper respiratory tract. The particles are removed by ciliary action which moves a film of mucus continuously from the lungs towards the oropharynx. Only particles < 4.5um can be deposited in the lungs as their inertial force is insufficient to impinge them on the mucous film. Droplet nuclei produced by airconditioning apparatus are within the size range capable of lung deposition.

There are a number of factors that interfere with the lungs' clearance mechanisms. People with such deficiencies comprise a significant proportion of the hospital population and are prone to nosocomial pulmonary infection. Hospital airconditioning systems constitute a health hazard to this group.

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LEGIONNAIRES' DISEASE

HISTORY OF LEGIONNAIRES' DISEASE

Following the American Legion's annual convention held in Philadelphia, Pennsylvania on July 21-24, 1976, 182 cases of a febrile illness were observed in those attending the meeting. By the time the epidemic had been notified to the Federal Public Health Department on Monday, August 2nd, 18 of the 29 deaths associated with the outbreak had occurred. (Fraser D.W., Tsai T.F., Orenstein W., 1977).

The Centre for Disease Control in Atlanta, Georgia, immediately began investigations to obtain epidemiological information and determine the aetiologic agent. Data and clinical features suggested that an infectious agent was responsible. However, within 48 hours most known agents capable of causing diseases of this severity had been excluded as possibilities. A battery of serological tests were performed for other microorganisms including bacteria, chlamydiae, fungi, mycoplasmas, parasites, rickettsiae and viruses. There were no positive results. (Lattimer G.L., Ormsbee R.A., 1981).

In January 1977, the aetiologic agent of Legionnaires' Disease (LD) was discovered by Dr. Joseph M^{c}Dade, who isolated a gram-negative bacillus from lung tissue of a fatal case of LD by techniques employing yolk sac inoculation, as used for the isolation of rickettsiae and chlamydiae.

Indirect fluorescent antibody tests using patients serum confirmed the isolate as the aetiologic agent (M^{c}Dade J.E., Shepard C.C., Fraser D.W., 1977).

Stored serum from two other outbreaks of respiratory disease occurring in 1965 (Washington DC) and 1968 (Pontiac Michigan) were also shown to react with the isolated organism (M^{c}Dade J.E., Shepard C.C., Fraser D.W., 1977).

Legionnaires' Disease Bacilli (LDB) were first isolated in 1947 by Jackson who isolated an organism from a sick guinea pig which had been inoculated with the blood of a patient with a febrile respiratory illness (M^{c}Dade J.E., Brenner D.J., Bozeman F.M., 1979). In 1959, Bozeman recovered an organism from the lung tissue of a patient who had died from what the U.S. Navy Medical personnel called "skin diver's disease". Both organisms had been stored since isolation and were identified as LDB by their cultural requirements, reaction with convalescent sera from LD patients and DNA hybridization reactions (M^{c}Dade J.E., Brenner D.J., Bozeman F.M., 1979).

Since the epidemic in Philadelphia, outbreaks have been noted throughout the world (Fischer-Hoch S.P., Smith M.G., Colbourne J.S., 1982; Dondero T.J., Rendtorff R.C., Mallison G.F., 1980; Merry D.J., Pitt J., Steele T.W., 1981). This was probably a result of heightened awareness by the medical profession coupled with an improvement in isolation procedures. The common denominator in many cases was a link with the airconditioning or hot water systems.

The examination of water obtained from cooling towers, humidifiers, hot water storage tanks and plumbing fixtures has frequently led to isolation of the LDB (Lattimer G.L., Ormsbee R.A., 1981).

The bacterium isolated by M^{c}Dade has been named Legionella pneumophila serogroup 1. A number of Legionella-like organisms have been isolated from cases of LD and two new genera, Tatlockia and Fluoribacter have been formed to accommodate these bacteria (Fallon R.J., 1983).

CLINICAL FEATURES AND DIAGNOSIS

The clinical diagnosis of LD is complicated by the broad range of manifestations. In its mildest form, the disease resembles influenza, however, it may present as a fulminant multisystem disease including pneumonia, diarrhoea, central nervous system involvement, hepatic dysfunction, shock and renal failure. The severe presentation of the disease has been termed the pneumonic form. A non-pneumonic form (also known as Pontiac Fever) occurs, with markedly different symptoms and characteristics. Pontiac Fever has a relatively mild clinical presentation and is distinguished from the pneumonic form by the absence of pneumonia and associated mortality. The attack rate is 95-100% compared with that for the pneumonic form of 0.1-3%. Underlying illness in Pontiac Fever has not been reported although it is a feature of the pneumonic form (Lattimer G.L., Ormsbee R.A., 1981). It has been suggested that Pontiac Fever is in fact a type of Hypersensitivity pneumonitis (Chapter 6) with concurrent exposure to LDB

(Rowbottom T.J., 1981).

The clinical and diagnostic features of the 1976 Philadelphia outbreak have been collated by Lattimer and Ormsbee and are presented here in summary.

Early symptoms of the Philadelphia epidemic were influenza-like with malaise, fever, chills and myalgia. Severe headaches were frequent and a moderate cough was evident although non-productive. Fever and chills progressed over several days with rigors commonly described. Confusion, delirium or lethargy developed in many patients. Abdominal pain and vomiting was occasionally noted although diarrhoea was a conspicuous initial symptom in a few patients.

Typically, several days after the onset of illness, the patient was seriously ill with a high temperature and prominent respiratory complaints often with mental confusion and diarrhoea. Four days was the average period between onset of symptoms and hospitalization. On admission, patients were usually dehydrated, tachycardia was often observed and patients were in respiratory distress with tachypnoea. Over half had temperatures above 38.9°C. Pulse, respiratory rate and temperature tended to be higher in those cases resulting in death.

On physical examination, abnormal findings were limited to the pulmonary system. Rales were heard in 80% of patients, rhonchi in 49%. while signs of consolidation were present in 21%.

Routine laboratory tests demonstrated a moderate leucocytosis with a leucocyte count of $10,000/mm^3$ in 83% of patients. The erythrocyte sedimentation rate (ESR) was > 66mm/hour in most patients.

Renal failure or insufficiency occurred in 15% of patients. Proteinuria was present in 25% of cases, leucocytes in the urine were uncommon. Serum electrolyte concentrations were generally normal for patients with uncomplicated pneumonia.

Pneumonia was the main clinical and radiographical finding. Chest x-rays showed interstitial infiltration and patchy distribution of consolidated areas indicative of atypical pneumonia. Arterial blood typically showed hypocapnea and respiratory alkalosis.

Extrapulmonary symptoms were evident in many cases and a retrospective analysis of 123 cases showed the following:

Gastrointestinal Tract

41% had diarrhoea, with 23% vomiting and 20% abdominal pain. In some instances the diarrhoea was of such severity that the clinical diagnosis was that of salmonella or shigella gastroenteritis. It is worth noting that a large number of these patients had been treated with antibiotics and diarrhoea could have been drug-associated. Acute liver damage was manifest by an increase in the serum glutamate oxaloacetate transaminase (SGOT), glutamate pyruvate trans-

aminase (GPT) and bilirubin, which occurred in 30% of cases. Liver function tests returned to normal after the illness without liver failure.

Central Nervous System

Headache, delirium, obtundation and hallucination were noted in hospital patients. Temperatures of 41.1°C and over occurred in six cases (hyperpyrexia of 41.1°C is usually caused by abnormalities in the central nervous system and not the result of systemic infection). Cerebrospinal fluid findings were normal in all who were tested. Neurological symptoms typically improved during the period of hospitalization.

Renal System

33% of patients exhibited casts in the urine, 44% had a high protein content while 16% had more than five leucocytes/high power field. 50% of patients demonstrated a microscopic haematuria. 30% had sodium levels <130 meq/l with 15% having high blood urea and creatinine levels. 14 patients developed oliguria or anuria, 11 of whom died (Lattimer G.L., Ormsbee R.A., 1981).

Differential Diagnosis

LD can be grouped amongst the atypical pneumonias. The agents responsible are principally, Mycoplasma pneumoniae, Chlamydia psittaci and Coxiella burnetii. Some toxic substances can produce similar symptoms and these include Paraquat, nickel carbonyl, carbonyl chloride (phosgene), ozone and cadmium (Lattimer G.L., Ormsbee R.A., 1981).

Usually, atypical pneumonias occur in clusters, at certain times of the year and in defined geographic areas. A history of recent travel, occupational exposure, exposure to birds, arthropods, wild and domestic animals and their products, and patients with similar symptoms can assist in the diagnosis (Lattimer G.L., Ormsbee R.A., 1981).

Organisms causing atypical pneumonias are usually acquired through inhalation of microbial aerosols whereas classical bacterial pneumonias are endogenous infections caused by organisms from the oral and nasal pharynx such as Streptococcus pneumoniae and Haemophilus influenzae. Diagnosis of atypical pneumonias is seldom achieved by the isolation of the organism but is most commonly established by retrospective analysis of paired serum samples. The production of antibodies may not be detected until late in the disease so that serodiagnosis is of limited use in guiding therapy (Lattimer G.L., Ormsbee R.A., 1981).

The comparitive features of pneumococcal, mycoplasmal and legionellal pneumonias reveal that encephalopathy is evident in a significant proportion of cases of LD but is unusual in the other pneumonias. Microscopic haematuria may occur in up to 50% of LD although it may also be observed to a lesser extent in mycoplasmal and pneumococcal pneumonias. SGOT levels of over 40IU/l is typical of LD and seen in 90% or more of patients compared with 30% for pneumococcal and 35% for mycoplasmal infections. Chest x-rays are similar in all instances (Lattimer G.L., Ormsbee R.A., 1981).

In cases of pneumonia with unexplained encephalopathy, haematuria and raised SGOT levels, LD is indicated (Lattimer G.L., Ormsbee R.A., 1981).

PATHOLOGY AND PATHOGENESIS

This section is a summary of studies by Lattimer and Ormsbee of the 1976 Philadelphia outbreak.

Death from LD is most commonly attributed to respiratory failure secondary to severe pneumonia. Pathology findings have noted that consistent and characteristic changes occurred only in the lungs. The interpretation of the results are affected by several factors:

i) in many of the fatal cases of LD there were underlying serious illnesses that may have contributed to or altered microscopic findings;

ii) treatment for organ system failure and the underlying illness can cause structural changes in lung tissue;

iii) secondary infections that occur in LD patients may compound the difficulties in interpreting pathological findings.

In a survey of 12 patients, none of whom were treated with either erythromycin or tetracycline, consolidation of air spaces was present in all cases with the lung weighing an average of 2200g (normal, 850g). Emphysema was observed in six cases. In ten cases the pneumonia was multilobular and usually the process was delineated by lobular septa.

In two cases, pulmonary consolidation was distinctly nodular and in two cases single abscesses were described which were not noticed radiographically. Fibrinous pleural inflammation was present in nine cases. Emphysema was not noticed.

The characterization of the pneumonia as lobular or lobar becomes difficult when the inflammation is extensive. Lobar distribution of LDB was evident in some cases which is compatible with a respiratory portal of entry. Diffuse spread of bacteria through the air spaces, however, is suggested in the non-lobular outlines in some large lesions which makes the exclusion of a haematogenous origin of infection impossible.

Microscopic Pathology

Microscopic examination of lung tissue showed acute inflammation that primarily involved the respiratory bronchi and alveoli, while sparing larger bronchioles, bronchi and the alveolar interstitium. Commonly, intra-alveolar consolidation was noted. A second pattern was evident which consisted of scattered areas of intra-alveolar exudate.

The microscopic lung findings have been classified under two categories. Most commonly, acute fibrinopurulent pneumonia was noted, consisting of dense intra-alveolar infiltrates of polymorphonuclear cells, macrophages, red blood cells and alveolar lining cells. The second tissue-reaction pattern was that of acute, diffuse alveolar damage, characterized by hyaline membranes, proliferative alveolar lining

cells, inconsistent interstitial mononuclear cell infiltrates and proteinaceous debris. A combination of both patterns is often noted.

Although LD is a multisystem disease, no consistent extrapulmonary lesions have been described. LDB have been identified in tissues outside the lung, in the spleen, kidney, bone and marrow by the direct fluorescent antibody stain. These reports have been exceptional cases.

The macroscopic and microscopic histological findings are characteristic but not pathognomonic of LD pneumonia. The presence of large numbers of intra-alveolar macrophages and monocytes has been noted as a frequent finding which is uncommon except in infections caused by rickettsial, chlamydial, viral and mycoplasmal agents. Other findings such as acute diffuse alveolar damage may be explained by the treatment or because of underlying illness.

The action of the organism in vivo is still a mystery. Local tissue invasion does not appear to be a major factor. The observation of inflammation and tissue injury in the absence of demonstrable organisms would suggest the elaboration of a toxin as the responsible agent. There is some evidence to suggest that this is the case, however, if a toxin is produced, it would appear to differ from that produced by other gram negative organisms (Lattimer G.L., Ormsbee R.A., 1981).

Cultural and Serological Methods

Ideally the laboratories function is to provide a rapid diagnostic test for LD. Culture techniques are unlikely to provide a result within five days even when the organism is present in pure growth, longer if the LDB has to be separated from a specimen contaminated with other microorganisms. Serological tests rely on the presence of antibodies which take at least 10 days to develop (Boquest A.L., Tosolini F.A., 1985). Direct fluorescent antibody tests provide a rapid result but positive reactions may be obtained with bacteria other than LDB (Edelstein P.H., M^{c}Kinney R.M., Meyer R.D., 1980). Some experimental work has been performed on the analysis of urinary antigen which can be detected in guinea pigs as early as four hours after inoculation with non-viable LDB (Farshy C.E. Feeley J.C., 1979). Despite these optimistic results, testing for legionella antigens has not been widely accepted.

Information derived from laboratory studies is a useful epidemiologic aid but generally only allows for retrospective diagnosis of LD.

Serological Methods

The Indirect Fluorescent Antibody Test (IFAT)

The method of choice for detecting antibodies to LD is the IFAT and is recommended in both America and England. The IFAT was vigorously tested and validated in 1981 using

sera from six outbreaks of LD including the 1976 Philadelphia epidemic (Wilkinson H.W., Cruce D.D., Broome C.V., 1981).

A significant result is one in which a fourfold rise in titre from the acute to the convalescent phase of illness is detected, or, in which a single titre of $\geq$256 occurs (Wilkinson H.W., Cruce D.D., Fikes B.J., 1979). The demonstration of IgM antibodies is of particular use in confirming diagnosis and a titre of $\geq$16 in a symptomatic patient is diagnostically useful (Harrison T.G., Taylor A.G., 1982).

The method of preparing the antigen for the IFAT has been the subject of some contention. The Centre for Disease Control in America uses a heat-killed antigen while the Central Public Health Laboratory in England claims their yolk-sac produced antigen eliminates cross reaction with Mycoplasma pneumoniae (Harrison T.G., Taylor A.G., 1982). Alternative methods have been described using variations of culture techniques, methods of killing and different strains of LDB (Benson R.F., Malcolm G.B., Pine L., 1983).

Generally, the IFAT is performed using Legionella pneumophila serogroup 1 or a polyvalent screening reagent containing antigens to serogroups 1-4 (Boquest A.L., Tosolini F.A., 1985). By 1983 at least 13 different serogroups had been isolated (Fallon R.J., 1983) which would suggest that cases of LD have been and are being missed.

One survey noted that if only monovalent antisera had been used then less than half of the total positive sera would have been detected (Wilkinson H.W., Reingold A.L., Brake B.J., 1983). Another report noted the need to test for both IgG and IgM antibodies as the production of both antibodies does not always occur. (Zimmerman S.E., French M.L.V., Allen S.D., 1982).

The availability of monoclonal antibodies in the future may eliminate some of the problems previously described. Monoclonal antibodies to LDB have been prepared, although not on a commercial scale, and have the advantage of being serogroup specific (Sethi K.K., Drücke V., Brandis H., 1983).

The Enzyme-Linked Immunosorbent Assay (ELISA)

The ELISA technique is more suitable than the IFAT for handling large numbers of specimens (Wreghitt T.G., Nagington J., Gray J., 1982). It has been claimed that the ELISA is more sensitive than the IFAT for the detection of antibodies to LDB, although not as specific (Sathapatayavongs B., Kohler R.B., Wheat L.J., 1982; Wreghitt T.G., Nagington J., Gray J., 1982; Elder E.M., Brown A., Remington J.S., 1983). There are a number of different methods available for the preparation of antigen along with a range of solid phase on which to coat it. The test is markedly influenced by this choice (Wreghitt T.G., Nagington J., Gray J., 1982).

The value of urinary antigen detection by ELISA, as a diagnostic tool for LD has been described (Farshy C.E.,

Feeley J.C., 1979; Berdal B.P., Farshy C.E., Feeley J.C., 1979). Antigen was detected in guinea pigs four hours after inoculation with non-viable Legionella pneumophila serogroup 1.

Radioimmuno Assay (RIA)

Urine from nine patients with LD caused by LDB serogroup 1 was readily identified from 245 other control specimens when examined for LDB antigen. The authors conclude by suggesting that RIA for urinary antigen is a useful method for the rapid diagnosis of LD (Kohler R.B., Zimmerman S.E., Wilson E., 1981).

Miscellaneous Techniques

Immunodiffusion: A survey comparing immunodiffusion to the IFAT showed eight out of 73 specimens positive for Legionella pneumophila serogroup 1 antibody using immunodiffusion with a correlation of 98.6% to the IFAT (Soriano F., Aguilar L., Garcés J.L.G., 1982).

Microagglutination: A microagglutination method using safranin-stained LDB as the antigen and reacting in 'V' well microtitre plates gave a 97% correlation with IFAT and took only 30 minutes to perform (Harrison T.G., Taylor., 1982).

Counterimmunoelectrophoresis (CIE): CIE tests have been described for the detection of antibodies to LDB. The specificity compared with the IFAT was found to be 93%

with 86.3% sensitivity (Holliday M., 1983).

Latex Agglutination: A latex agglutination method for the detection of antigens to Legionella pneumophila was performed on 161 control urines with only a single false positive result. 82% of urine specimens previously proven positive for antigens to Legionella pneumophila were shown to be positive by this technique (Sathapatayavongs B.,

Direct Staining Techniques

Although the LDB has a cell wall structure classifying it as a gram-negative rod it stains poorly by Gram stain. Smears prepared from cultures may be stained by the 'half-a-Gram' method. This stain is completed following the addition of iodine and is particularly useful if compared with an ordinary Gram stain (Lattimer G.L., Ormsbee R.A., 1981). Paraffin sections and exudate smears may be stained by the Giménez technique (Giménez D.F., 1964), however, for best results, paraffin sections should be stained by a silver impregnation method such as Dieterle's (Dieterle R.R., 1927).

The direct fluorescent antibody test (DFA) is a useful adjunct to the culture and rapid diagnosis of LD. It can indicate the presence of LDB in clinical and environmental specimens although interpretation of results has to be made with care. Some bacteria such as staphylococci and streptococci may fluoresce due to the presence of pre-existing

antibodies in the animal in which the antiserum was raised (Feeley J.C., Gorman G.W., 1980). Pseudomonas fluorescens and Bacteroides sp. have also been shown to produce false positive results (Edelstein P.H., M^{c}Kinney R.M., Meyer R.D., 1980). If antisera is produced using Freund's adjuvant (which contains mycobacteria) then mycobacteria may fluoresce (Thomason B.M., Harris P.P., Lewallen K.R., 1979).

CULTURE METHODS

M^{c}Dade in 1977 originally isolated LDB by inoculating macerated lung tissue into fertilized chicken eggs whilst testing for the presence of rickettsiae (M^{c}Dade J.E., Shepard C.C., Fraser D.W., 1977). Following this a medium for the isolation of Neisseria gonorrhoeae was found to support the growth of LDB. The medium was a Mueller-Hinton agar base incorporating 1% haemoglobin and 1% IsoVital-X (BBL, Microbiology Systems, Cockeysville, Md). IsoVital-X contains L-cysteine hydrochloride, adenine, p-aminobenzoic acid, L-cystine, glucose, nicotinamide adenine dinucleotide, ferric nitrate, glutamine hydrochloride, guanine hydrochloride, thiamine hydrochloride and vitamin B12. It was found that only the L-cysteine hydrochloride was necessary for the growth of LDB (Feeley J.C., Gorman G.W., Gibson R.J., 1979) whilst ferric pyrophosphate has since replaced haemoglobin.

The egg yolk inoculation method and modified Mueller-Hinton agar were both used for the isolation of LDB from material uncontaminated with other bacteria. Enrichment media presently being used includes Feeley-Gorman agar (Feeley J.C., Gorman G.W., Weaver R.E., 1978) and charcoal yeast extract agar (CYE) (Feeley J.C., Gorman G.W., Gibson R.J., 1979). Specimens such as sputum and environmental material cannot be processed on this media because of bacterial overgrowth.

Separation techniques require concentration of the specimen first, to increase the isolation rate. Water specimens are concentrated through centrifugation (Flier-mans C.B., Cherry W.B., Orrison L.H., 1979) or filtration (Orrison L.H., Cherry W.B., Milan D., 1981). Separation techniques initially were achieved through the intra-peritoneal inoculation of guinea pigs (M^{C}Dade J.E.,1979). Recently, it has been determined that sacrifice of the animal at day 3 following inoculation, increases the likelihood of isolating LDB from the peritoneal fluid, spleen and heart blood (Leinbach E.D., Winkler H.H., 1983).

Guinea pig inoculation is being used less frequently following the improvement of selective media and techniques which are claimed to be as effective. Selective media has been prepared using a variety of antibiotic combinations (Edelstein P.H., Wadowsky R.M., Yee R.B., 1981) in a buffered CYE base (Pasculle A.W., Feeley J.C., Gibson R.J., 1980).

Selective techniques are being used in conjunction with selective media to isolate LDB. The media advocated by Edelstein, BMPA∝ (Edelstein P.H., 1982) is recommended to be used with an acid (pH 2.2) pre-treatment technique (Buesching W.J., 1983; Bopp C.A., Sumner J.W., Morris G.K., 1981). Another selective procedure involves the addition of antibiotics to the specimen as a negative enrichment technique (Thorpe T.C., Miller R.D., 1980).

Biological isolation of LDB has been achieved through the harvesting of amoebae. The LDB exist as an intracellular parasite in the protozoa (Rowbottom T.J., 1983).

IDENTIFICATION

LDB grows best in an atmosphere of 2.5% CO_2 in air at a temperature of 35°C. Plates are incubated for two weeks and examined daily using a dissecting microscope. Generally, Legionella pneumophila requires 3-5 days to grow and has a typical 'cut-glass' texture on CYE. On Feeley-Gorman media, colonies produce a distinct brown darkening of the surrounding agar (Feeley J.C., Gorman G.W., 1980).

Gram negative bacilli resembling Legionella pneumophila in colonial morphology and which grow on media containing L- cysteine hydrochloride, but not on media lacking this substance are probably LDB (Feeley J.C., Gorman G.W., 1980).

Biochemical methods are not very useful in identifying LDB because most tests are negative. The few exceptions are, catalase, oxidase, gelatinase and urease, however, even these tests may be weakly positive or even negative and on their own cannot offer definitive identification (Feeley J.C., Gorman G.W., 1980).

Gas liquid chromatography is a useful method for differentiating LDB from other bacteria. LDB contain large amounts (>77%) of branched chain fatty acids, which represents a unique profile. In conjunction with the DFA test, a complete identification to species level can usually be achieved (Feeley J.C., Gorman G.W., 1980).

DNA hybridization studies can be performed on organisms that have unusual characteristics when tested by conventional methods. This technique is highly specialized and time consuming. It is used principally on isolates that have a cellular fatty acid profile compatible with LDB and which do not stain with DFA conjugates (Feeley J.C., Gorman G.W., 1980).

The incorporation of 0.001% bromocresol purple and 0.001% bromothymol blue (Vickers R.M., Brown A., Garrity G.M., 1981) and also 0.01% aniline blue (Holmes R.L., 1982) to buffered CYE has had some success with the rapid differentiation of Legionella sp. through the variable uptake of stains.

Immunoperoxidase techniques are useful in the identification of LDB from paraffin sections (Boyd J.F., M^{c}Williams E., 1982).

Electron microscopy has shown some differences in the ultrastructure of different Legionella sp., but not sufficient to enable species identification (Hébert G.A., Calloway C.S., Ewing E.P., 1984).

ANTIBIOTIC THERAPY

No controlled trial of antibiotics used in LD has been performed or is likely to be performed. The antibiotic regimens for this disease have been determined through data derived from epidemics and sporadic cases. Laboratory studies have pointed towards erythromycin and rifampicin being effective in low doses whilst showing that tetracycline derivatives have little activity against LDB. These results correlate with clinical observation for erythromycin and rifampicin, however, tetracycline has been shown to be effective in treating LD (Miller A.C., 1981).

In a cluster of cases of LD from Vermont (U.S.) in 1977, mortality was significantly reduced in the erythromycin-treated group. Two out of 27 patients treated with erythromycin died compared with six deaths from 13 patients not treated with erythromycin. Reports however, showed that in some cases, patients failed to respond to erythromycin even when LD was diagnosed and erythromycin commenced early in the disease (Miller A.C., 1981).

The Philadelphia outbreak of 1976 provides one of the best controlled surveys available because the cause of the disease was not known at the time and the use of antimicrobial agents was totally empiric. Mortality rates with erythromycin was 10%, tetracycline - 11%, penicillin - 20%, ampicillin - 24%, chloramphenicol - 30%, aminoglycosides - 36% and cephalosporins - 41%. Treatment with erythromycin or tetracycline was not uniformly successful and progressive disease and death occurred despite therapy that is now accepted as the treatment of choice (Lattimer G.L., Ormsbee R.A., 1981).

Methods available to the laboratory for testing the efficacy of antibiotics to LD have not always been in agreement with data obtained from clinical studies. The breakpoint minimum inhibitory concentration (MIC) technique using agar dilution of antibiotics on modified Mueller-Hinton agar provided the following results:-

Sensitive: rifampicin, cefoxitin, erythromycin, gentamicin, tobramycin, chloramphenicol, ampicillin, penicillin, cotrimoxazole.

Intermediate: tetracycline, cefamandole.

Resistant: vancomycin (Thornsberry C., Baker C.N., Kirven L.A., 1978).

As LDB can live both intracellularly and extracellularly it would appear more reliable to test antibiotics in a living

system. Animal models have not proven successful though because of the difficulty in finding a suitable subject and also because the mode of inoculation should be through the inhalation of aerosols for the model to parallel the actual mode of transmission of the disease (Miller A.C., 1981).

A study testing the susceptibility of Legionella pneumophila to ten antibiotics through the inoculation of embryonated eggs has shown results at variance to the agar dilution method. The minimum dose of antibiotic preventing death from infection was: rifampicin - 0.02mg, gentamicin - 0.25mg, streptomycin - 0.39mg, erythromycin - 0.62mg, sulfadiazine - 1.56mg, chloramphenicol - 2.50mg, cephalothin - 20.0mg. Oxytetracycline in the largest amount tested, 5.0mg, protected 80% of the embryos from death, while as little as 0.31mg delayed death. Chlortetracycline and ampicillin were ineffective (Lewis V.J., Thacker W.L., Shepard C.C., 1978).

It would appear that the patient's prognosis is related to the choice and expediency with which antibiotics are administered and the severity of underlying disease. It is likely that erythromycin benefits patients treated within three to five days of the start of the illness. Prior treatment with corticosteroids may adversely affect antibiotic therapy (Lattimer G.L., Ormsbee R.A., 1981).

The recommended therapeutic approach in patients with suspected or proven LD pneumonia is maximum dose, intra-

venous erythromycin for a period of three weeks. Although tetracycline appears to be as efficient in clinical studies there is less data to suggest it is the drug of choice. In critically ill patients not responding to either erythromycin or tetracycline therapy, the addition of rifampicin should be considered. There is little data available for the recommended therapy of LDB other than Legionella pneumophila, however, in vitro studies suggest antibiotic sensitivity patterns are similar (Lattimer G.L., Ormsbee R.A., 1981).

The possibility of secondary infection must not be overlooked, especially if the patient's condition appears to be improving, then deteriorates (Lattimer G.L., Ormsbee R.A.,1981).

EPIDEMIOLOGY

Since the cause of LD was first established, following the Legionnaires' convention in Philadelphia in 1976, there have been numerous reported cases, including outbreaks from countries in Europe (Fischer-Hoch S.P., Smith M.G., Colbourne J.S., 1982; Kurtz J.B., Bartlett C.L.R., Newton V.A., 1982) America (Stout J., Yu V.L., Vickers R.M., 1982; Arnow P.M., Chou T., Weil D., 1982; Dondero T.J., Rendtorff R.C., Mallison G.F., 1980) and Australasia (Bettelheim K.A., Metcalfe R.V., Sillars H., 1982; Merry D.J., Pitt J., Steele T.W., 1981). A brief look at several such outbreaks is useful in understanding the typical epidemiological pattern.

In 1981, nine cases of hospital acquired pneumonias occurred in renal transplant patients at the Veteran's Administration Medical Centre, Pittsburgh, Pennsylvania USA. The aetiologic agent was originally known as the Pittsburgh Pneumonia Agent (PPA) and is now called Tatlockia micdadei. Additionally an unspecified number of LD cases caused by Legionella pneumophila occurred. Both organisms were isolated from hot water storage tanks, showerheads, mixing valves and taps servicing 20 of the hospital's wards including the intensive care unit (Stout J., Yu V.L., Vickers R.M., 1982).

The Kingston Hospital in Kingston, Surrey U.K. has had continual problems with the contamination of their air-conditioning and hot water systems with Legionella pneumophila. In August 1981, a 37 year old male patient in previously good health developed LD following a seven day stay in hospital for routine investigations. The development of pneumonia coincided with the use of a hot water cylinder which had previously been closed down over the summer period. The slurry in the bottom of an adjacent hot water cylinder, also shut down for summer, revealed the presence of 5.4×10^8 colony forming units (cfu) of Legionella pneumophila per litre (Fischer-Hoch S.P., Tobin J.O'H., Nelson A.M., 1981; Fischer-Hoch S.P., Smith M.G., Colbourne J.S., 1982).

Thirteen people who had visited a hotel complex in Eau Claire, Wisconsin USA. in1979 developed LD. Water

samples collected from a cooling tower situated on top of the building and a nearby puddle (upwind of the cooling tower) were positive for Legionella pneumophila. Smoke-tracer studies showed that aerosolized exhaust from the cooling tower could be transmitted via a chimney to a meeting room in which all 13 had visited. The patients had an average age of 67 and when matched with control subjects were shown to be more likely to have an underlying chronic illness. These included diabetes, congestive heart failure, obstructive pulmonary disease and malignant disease. Four patients died (Band J.D., La Venture M., Davis J.P., 1981).

The last case-study demonstrates the classic development of an infectious disease from an epidemiologic perspective. There are three criteria which must be fulfilled, a reservoir for the aetiologic agent; a mode of transmission; the presence of a susceptible host. The reservoir in this case was the cooling tower, with airborne transmission down the chimney being demonstrated by air-tracer studies. The thirteen affected people had a higher incidence of chronic illness than the control group and were in the elderly age bracket.

Data compiled from numerous outbreaks has enlarged the understanding of LD, the aetiologic agent and its epidemiologic features. A brief account of the reservoir, transmission and host follows.

Reservoir

Most cases of LD can be traced to a building in which, if further investigation is made, the LDB will be isolated from either the airconditioning or hot water system or both. Both of these systems contain apparatus holding large volumes of water which provides an ideal habitat for LDB (Schofield G.M., Wright A.E., 1984). In summer, cooling towers often reach a temperature of 32-42°C and maintain this for long periods (Wadowsky R.M., Yee R.B., Mezmar L., 1982). If the cooling tower has been out of operation during the winter months, then recommissioned for summer use there is a greater likelihood of contamination. Proliferation of LDB is encouraged in this situation due to the build-up of organic and inorganic matter (Fischer-Hoch S.P., Smith M.G., Colbourne J.S., 1982).

In some outbreaks of LD there is a history of recent excavation in the vicinity (Thacker S.B., Bennett J.V., Tsai T.F., 1978). It seems likely that LDB in the soil represents the original source of contamination (Fliermans C.B., Cherry W.B., Orrison L.H., 1979). It has been shown that LDB exist in potable water although their viability is in doubt as they have only been demonstrated by DFA techniques and have not been isolated (Tison D.L., Seidler R.J., 1983).

The summer seasonality of LD has been noted and is reflected in the density of LDB isolated from environmental sources in a year round study (Fliermans C.B., Cherry W.B., Orrison L.H., 1981). In some circumstances, conditions occur

throughout the year which encourage the growth of LDB. This is the case in some mental institutions where the hot water temperature is lowered to 40-45°C. in order to reduce the risk of patients scalding themselves. Considerable problems have occurred in institutions which have opted for these temperatures (Plouffe J.F., Webster L.R., Hackman B., 1983).

Transmission

There is considerable difficulty in conclusively determining the mode of transmission in epidemic LD. There is, however, overwhelming evidence that LDB is transmitted in an airborne fashion (Fraser D.W., Tsai T.F., Orenstein W., 1977; Band J.D., La Venture M., Davis J.P., 1981). Even in outbreaks from buildings without an airconditioning system, an airborne mode of spread has been suggested. One such case, occurring in 1965, was thought to be associated with the digging of irrigation trenches. 81 cases of respiratory disease occurred in patients at the St. Elizabeth Hospital, Washington D.C. Attack rates increased with proximity to the excavations and also with patients sleeping close to open windows (Thacker S.B., Bennett J.V., Tsai T.F., 1978).

The 1968 outbreak of Pontiac Fever in Pontiac, Michigan USA, was convincingly demonstrated to have an airborne mode of spread. This was considered because the attack rate was 95%. The airconditioning system was suspected of being the source, as infection mirrored its use. Defects in the airconditioning system were also noted. Experimental confirmation of airborne spread was achieved by recovering Legionella

pneumophila from lung tissue of guinea pigs exposed to air in the Pontiac building (Glick T.H., Gregg M.B., Berman B., 1978; Fraser D.W., 1980).

Person to person transmission of LD has not been demonstrated despite the mass of data collected from families and contacts of people acquiring LD (Yu V.L., Zuravleff J.J., Gavlik L., 1983). It seems that if it occurs it must be exceedingly rare (Lattimer G.L., Ormsbee R.A., 1981). Seroreactivity amongst family contacts has not been demonstrated. (Marks J.S., Tsai T.F., Martone W.J., 1979).

Study of airborne transmission of Legionella pneumophila would be assisted if methods were available to recover the organism from the air. The use of sentinel guinea pigs was successful in the Pontiac outbreak but has not been so for pneumonic LD. Air sampling methods have not been successful although impingement into a liquid medium may prove effective (Fraser D.W., 1980).

Susceptible Hosts

The infectivity rate of the non-pneumonic form of LD (Pontiac Fever) is 95-100% which discounts the presence of risk factors (Glick T.H., Gregg M.B., Berman B., 1978). The pneumonic form of LD has an infectivity rate of $<3\%$ with predisposing factors being evident in some but not all cases (Lattimer G.L., Ormsbee R.A., 1981).

The average age for acquiring LD is 55-60, with cases being rare amongst children. Cigarette smoking increases the likelihood of infection by 4.2 times. Alcohol consumption also appears to be a predisposing factor. LD occurs in males at a rate 2.25-2.4 times higher than in females (Lattimer G.L., Ormsbee R.A., 1981). Construction workers have a higher infection rate than other sections of the community, presumably because of their involvement with excavations and the association of LDB with soil (Harkness J., Wright R., Hayes J., 1978).

12 cases of LD occurred at the Kingston Hospital, Surrey U.K. Eight of the patients were either immunosuppressed or debilitated. The remaining four cases were in previous good health and the reason suggested for their infection was exposure to a high concentration of LDB (Fischer-Hoch S.P., Tobin J.O'H., Nelson A.M., 1981). Nosocomial LD infections have occurred in healthy patients (Fischer-Hoch., Smith M.G., Colbourne J.S., 1982) but are more frequently associated with patients who are immunocompromised. Out of 65 cases of LD occurring at the Wadsworth V.A. Hospital, U.S. 61 (94%) had significant underlying disease and 27 (44%) were receiving immunosuppressive therapy (Kirby B.D., Snyder K.M., Meyer R.D., 1980). The Pittsburgh Pneumonia Agent, Tatlockia micdadei has only been isolated from patients hospitalized for organ transplants (Pasculle A.W., Feeley J.C., Gibson R.J., 1980).

Information regarding the epidemiological aspects of LD will be improved by the standardization of serological pro-

cedures along with an improvement in isolation techniques (Lattimer G.L., Ormsbee R.A., 1981).

CONTROL

The bacteria causing LD have been isolated from air-conditioning (Kurtz J.B., Bartlett C.L.R., Newton V.A., 1982) and hot water systems (Wadowsky R.M., Yee R.B., Mezmar L., 1982) and have been seen by DFA tests in domestic water supplies (Tison D.L., Seidler R.J., 1983). These sites are the reservoir of infection. Control measures are indicated, to prevent multiplication of LDB and resultant infection.

There is a general lack of information regarding the prevention and control of LDB contamination in airconditioning systems (Lattimer G.L., Ormsbee R.A., 1981). The studies performed in this area have, to some extent, contradicted each other (Soracco R.J., Gill H.K., Fliermans C.B., 1983) and have indicated that laboratory tests do not necessarily reflect the true situations found in airconditioning systems (Grace R.D., Dewar N.E., Barnes W.G., 1981). The investigation of cooling tower biocides has been complicated by the fact that there is no uniform technique being applied to test their efficacy. Some workers have used the US. Environmental Protection Agency (USEPA) guidelines (Skaliy P., Thompson T.A., Gorman G.W., 1980; Grace R.D., Dewar N.E., Barnes W.G., 1981) however, they have not standardized biocide concentrations. Other workers have tested biocides directly on contaminated airconditioning systems (Kurtz J.B., Bartlett C.L.R., Newton V.A., 1982). The variation of methods used to test biocides

has resulted in difficulty in drawing conclusions from the data.

It is not sufficient to merely test LDB for their susceptibility to biocides, to obtain results applicable to actual situations. Biocides can produce chemical contamination of air ducted to rooms within a building (ACOA Report, 1984). The LDB appear to rely upon a complexity of factors for their survival. It has been shown that they can survive within amoebae and may be parasitically associated with this organism (Tyndall R.L., 1932). There is evidence to suggest that LDB have an affiliation with blue green algae (cyanobacteria) (Tison D.L., Pope D.H., Cherry W.B., 1980). Further association with inorganic material such as rust has been recorded (Potvliege C., Glupcynski Y., Labbe M., 1984).

The airconditioning and hot water system may contain an environment suitable for the growth of algae, protozoa, bacteria and other microorganisms in a variety of symbiotic relationships. Added to this may be salt deposition, debris and bacterial slime (Kurtz J.B., Bartlett C.L.R., Newton V.A., 1982; Miller R.P., 1979). These conditions need to be taken into account when testing the efficiency of biocides.

General Control Methods

The control methods that are advocated for general airconditioning maintenance include:- regular cleaning or replacement of filters; cooling towers, condenser trays and water trays should be checked regularly for signs of sludge,

algae and rust; the water trays should be cleaned every 2-3 weeks; air washers, humidifiers and eliminator plates should be cleaned regularly, cooling towers and ducting should be cleaned annually (ACOA Report, 1984), regular bleeding of accumulated organic and inorganic waste from evaporative condensers and cooling towers; chemical treatment (Kurtz J.B., Bartlett C.L.R., Newton V.A., 1982; Miller R.P., 1979); addition of chlorine at 5 parts per million (ppm); anti-scaling compounds and algicides (Editorial, 1981).

Biocides

The efficiency of biocides may well be affected by the degree of organic and inorganic contamination present in the system. In laboratory trials the biocide combination N-alkyl dimethyl benzyl ammonium chloride and bis (tri-n-butyltin) oxide was able to kill Legionella pneumophila at a concentration of 2 ppm (Grace R.D., Dewar N.E., Barnes W.G., 1981). A similar combination, dimethyl-didecyl ammonium chloride and tributyl tin oxide tested at a concentration of 10 ppm on an actual cooling tower contaminated with Legionella pneumophila, algae, protozoa, bacteria and inorganic material, was ineffective (Kurtz J.B., Bartlett C.L.R., Newton V.A., 1982).

A study using methods approved by the USEPA concluded that two biocides approved for use in cooling towers, are effective in the control of Legionella pneumophila. They are, chlorine, 2, 2-dibromo-3-nitrilopropionamide and a compound containing didecyl dimethyl ammonium chloride and

isopropanol (Skaliy P., Thompson T.A., Gorman G.W., 1980). The authors of this report are planning further studies on the efficiency of these two biocides in naturally contaminated cooling towers.

Chlorination and Water Temperature

The elimination of LDB from airconditioning and hot water systems has proven to be difficult (Wadowsky R.M., Yee R.B., Mezmar L., 1982; Best M., Stout J., Muder R.R., 1983; Plouffe J.F., Webster L.R., Hackman B., 1983). The bacterium is able to tolerate chlorine levels generally achieved in potable water (Kuchta J.M., Stales S.J., M^{c}Namara A.M., 1983). Water temperature and pH appear to be directly related to the efficiency of chlorine in killing Legionella pneumophila. It has been shown that at 21^{o}C, pH 7.6 with 0.1mg of free chlorine residual per litre of water, a 99% kill of Legionella pneumophila was achieved within 40 minutes compared with less than one minute for Escherichia coli. At pH 7.0, 99% of Legionella pneumophila were killed in less than ten minutes, while at pH 6.0, 99% kill was achieved in less than five minutes. The required contact time was twice as long at 4^{o}C than it was at 21^{o}C (Kuchta J.M., Stales S.J., M^{c}Namara A.M., 1983).

Hot water systems that are operated at temperatures less than 54^{o}C are prone to contamination with LDB, whilst those maintained above 58^{o}C do not appear to become contamin-

ated (Plouffe J,F., Webster L.R., Hackman B., 1983). This temperature control phenomenon has been used to minimise the level of contamination in hot water systems. It also explains the higher incidence of LD in summer months where the bacterium enjoys conditions which allow it to proliferate in cooling towers.

Raising the temperature of hot water to between 60°C and 77°C for 72 hours at a time, every 1-2 months, in conjunction with the flushing of taps for 30 minutes, resulted in a decline in the concentration of Legionella pneumophila and Tatlockia micdadei that had been contaminating a hot water system. The incidence of nosocomial LD infections was also reduced. The free chlorine concentration was between 0.1ppm and 0.5ppm (Best M., Stout J., Muder R.R., 1983).

It is both difficult and expensive to eliminate LDB from a hot water system or cooling tower once it is contaminated (Best M., Stout J., Muder R.R., 1983). In hot water systems that have always been maintained above 58°C contamination has not occurred (Plouffe J.F., Webster L.R., Hackman B., 1983). The implication here, is that expense, inconvenience and risk of infection can be avoided if a system is prevented from becoming contaminated in the first instance. If this is the case then control of LDB from cooling towers and other conditioning structures can be achieved by discouraging the bacteria from colonization through the general maintenance measures previously described.

In buildings that are contaminated with airborne LDB the use of ultra violet (UV) light may be useful in its control, although this method has not been used specifically for LDB. Trials using UV air disinfection for the upper air in a room have been successful for controlling the transmission of influenza virus (Riley R.L., 1974). Aerosolized test organisms have been effectively removed from upper air through UV irradiation. The efficiency is to some extent dependent on the temperature gradient in the room, which controls the mixing rates between the upper and lower air. the practice of UV air disinfection is regarded as being safe for human health (Riley R.L., Permutt G., Kaufman J.E., 1971).

Preventative techniques to discourage the colonization of airconditioning systems with LDB appears to be more satisfactory than trying to eradicate the bacteria after contamination. On occasions when LDB have contaminated the airconditioning system, disinfection has not been achieved and expensive apparatus has had to be abandoned (Best M., Stout J., Muder R.R., 1983).

SUMMARY

LD in its fulminant pneumonic form is a life threatening disease requiring rapid diagnosis and aggressive treatment. Fortunately, the disease has a low infectivity rate with <3% of exposed people actually demonstrating symptoms.

Those succumbing to the disease, usually have a predisposing factor such as renal transplantation, steroid therapy or underlying disease, or fall into the 'at risk' category: those with a high alcohol intake, cigarette smokers and the aged.

The prevalence of LDB in airconditioning systems and in the environment suggests that contact with the organism occurs frequently, without evidence of serious disease. Possibly, influenza-like symptoms occur in the healthy person, but is passed off as a mild viral episode.

The diagnosis of LD is difficult from a clinical viewpoint because it is hard to distinguish from other atypical pneumonias. Culture techniques have improved considerably, also the DFA test may assist in achieving a diagnosis. The most reliable diagnostic technique is the demonstration of antibody production, principally by the IFAT but the ELISA test is of similar reliability. The presence of urinary antigen may allow for a rapid diagnosis, although for reasons not clear, this technique has not achieved popularity. Two factors for consideration are, that serological diagnosis is useful as a retrospective means of analysis but less so as an immediate diagnostic tool and secondly, that all serotypes of Legionella, Tatlockia and Fluoribacter need to be tested to conclusively exclude LD. This would be impractical in all but reference laboratories.

It seems unlikely that the LDB is an organism that has just been noticed because it has recently mutated or evolved. More likely, is that the changing technology of man has allowed it to adopt a niche in airconditioning and hot water systems where it can multiply and cause infection. The study of the bacterium, its pathogenic features, epidemiology and laboratory characteristics will enable the microbiologist and engineer to combine forces in reducing the incidence of LD.

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CHAPTER 6

HYPERSENSITIVITY PNEUMONITIS

Legionnaires' disease (LD) and hypersensitivity pneumonitis (HP) comprise the two major diseases acquired from microbiologically contaminated airconditioning systems. Whilst LD is an invasive bacterial disease, HP is an allergic disorder. It affects the peripheral, gas exchanging region of the lungs, and is caused by the deep inhalation of relatively large amounts of any of a number of organic dusts (Marinkovich V.A., Hill A., 1975). This organic material may be acquired from a variety of sources including airconditioning systems. Thus HP is a general term and not exclusively applied to airconditioners.

In the context of airconditioning systems, it becomes evident from the literature that HP is not a clearly defined disorder. A range of symptoms may be experienced and HP would more accurately be described as a syndrome. Two relatively distinct disease entities emerge from this. The first is an acute febrile condition that is referred to by Banaszak as the acute form of HP (Banaszak E.F., Thiede W.H., Fink J.N., 1970) and by other workers as humidifier fever (Cockcroft A., Edwards J., Bevan C., 1981) or extrinsic allergic alveolitis (Van Assendelft A., Forsén K.O., Keskinen H., 1979). The second disease is described by Banaszak as the insidious form of HP and is chronic in its course without producing fever. It is variously named by other workers

as; humidifier lung, hypersensitivity alveolitis, hypersensitivity lung disease, interstitial lung disease and humidifier disease.

In this thesis the nomenclature used by Banaszak will be employed (hypersensitivity pneumonitis acute form and hypersensitivity pneumonitis insidious form).

History of hypersensitivity pneumonitis

Pulmonary disease caused by hypersensitivity to organic dusts was first reported by Ramazzini in 1713 (Weiss N.S., Soleymani Y., 1971). He described a pneumonia-like illness in individuals working with cereal grains that were not properly dried before storage. In this century, descriptions of farmers' lung disease, maple bark lung, bagassosis and other diseases coming under the broad heading, hypersensitivity pneumonitis, have been documented (Schatz M., Patterson R., 1983).

In 1970, a report was published which involved four of 27 office workers. Two developed acute interstitial pneumonia (HP acute form) and two developed insidious restrictive lung disease (HP insidious form). All four exhibited serum precipitins to an extract prepared from the office airconditioner. A bronchial challenge to that extract resulted in one of the subjects demonstrating a reproduction of his symptoms (Banaszak E.F., Thiede W.H., Fink J.N., 1970). This was the first recorded case of hypersensitivity pneumonitis acquired from an airconditioning system.

Since the report of Banaszak there have been over 100 documented accounts of HP having been acquired from office, home and car airconditioning systems (Marinkovich V.A., Novey H.S., 1983).

Clinical and laboratory aspects

There are a number of clinical features common to both forms of HP. Dypsnoea on exertion is the most obvious symptom and occurs in all cases to some extent. Other symptoms include; chest tightness, headache, fatigue, muscle aches, cough and nausea. Anorexia and weight loss may occur if exposure to the allergen persists over a long period of time (Friend J.A.R., Gaddie J., Palmer K.N.V., 1977).

Table 1

Differential features of the two forms of hypersensitivity pneumonitis (Cockcroft A., Edwards J., Bevan C., 1981; Fink J.N., Banaszak E.F., Barboriak J.J., 1976)

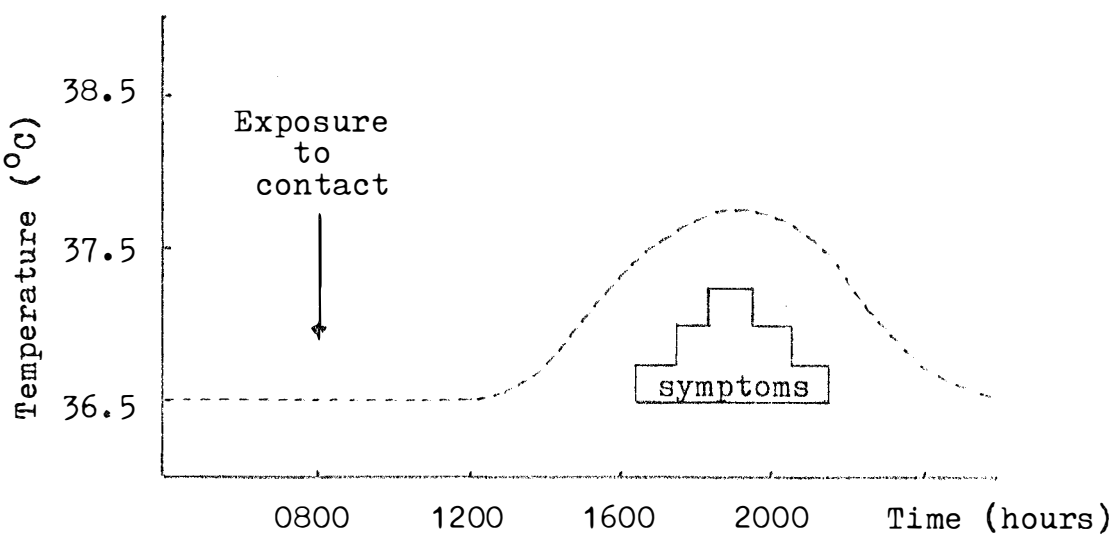
	Acute Form	Insidious Form
Periodicity	Most severe on initial contact, tending to improve if exposure to the allergen persists.	Increasing in severity with exposure to the allergen.
Duration	Acute	Chronic
Fever/Chills	Yes	No

HP acute form typically presents as a condition that affects the individual 6-8 hours after exposure to the allergen. With continued exposure, the symptoms tend to decrease if not disappear. The symptoms recur after a short break away from the allergen such as at the weekend or after holidays. Consequently, in a normal working week, symptoms are experienced late Monday afternoon and improve as the week progresses.

HP insidious form, however, for reasons unknown is a more serious condition that results in progressively decreasing lung function with exposure to the allergen. There is no evidence of the acute form progressing to the insidious form.

Figure 1

A typical pattern of response to allergens in HP acute form (Banaszak E.F., Thiede W.H., Fink J.N., 1970



HP insidious form is more difficult to diagnose because of its gradual onset and chronic course. This disease may lead to irreversible lung damage and if exposure to the allergen is not discontinued, then death may result (Schlueter D.P., 1974).

Tests used in the diagnosis of HP

Chest Xray examination of patients with HP acute form often show no abnormalities (Cockcroft A., Edwards J., Bevan C., 1981), however, cases have been reported where radiologic change, including diffuse infiltrates, have been demonstrated (Banaszak E.F., Thiede W.H., Fink J.N., 1970).

Radiologic results from cases of HP insidious form vary depending on the length of exposure. After short-term exposure the chest Xray may be normal, however, with longer exposure two patterns emerge. Firstly a small, poorly defined, uniform, mostly discrete, diffuse nodulation occurs. These are equally distributed in both lungs and may measure up to several millimetres in diameter. A second pattern, with similar distribution and intensity to the first is manifest by a diffuse, soft, stringy or patchy interstitial infiltrate (Schlueter D.P., 1974).

Lung function tests provide results that are similar for both forms of HP. Reduced vital capacities and flow rates are typical features. Arterial blood gas analyses demonstrate subnormal oxygen and carbon dioxide tensions with alkalosis (Fink J.N., Banaszak E.F., Barboriak J.J., (1976).

Routine pathology tests generally show a normal full blood count with a normal differential cell count although a mild leucocytosis with a left shift may be evident. Eosinophilia is not a feature but may be observed because of a concomitant allergic condition (Weiss N.S., Soleymani Y., 1971). The erythrocyte sedimentation rate (ESR) may be significantly raised in HP acute form but not in the insidious form (Schatz M., Patterson R., 1983). Urine analysis is normal. Rheumatoid factor in high titres has been noted in some cases (Banaszak E.F., Thiede W.H., Fink J.N., 1970).

Challenge studies with extracts of material suspected of being allergenic can offer strong evidence towards the diagnosis. Intradermal testing characteristically produces a red, tender swelling at the site of infection (Marinkovich V.A., Hill A., 1975). Aerosol insufflation of allergenic material results in fever and chills, an elevated leucocyte count and pulmonary function changes (Fink J.N., Banaszak E.F., Barboriak J.J., 1976)

The diagnosis of HP requires considerable effort and involves the compilation of a detailed history plus lung function and other laboratory tests. Challenge studies may be useful in confirming the diagnosis although they are not without hazards (Fink J.N., Banaszak E.F., Barboriak J.J., 1976). HP must be differentiated from asthma, allergic bronchopulmonary aspergillosis, infectious pneumonia, byssinosis and interstitial lung disease (Schatz M., Patterson R., 1983).

Treatment

Removal of the patient from contact with the allergen is the primary consideration in treatment. If this is not done, the patient may progress to irreversible lung damage and possibly death. Therapy with steroids (eg. prednisolone) usually results in rapid improvement of pulmonary function although full lung function may never be restored (Marinkovich V.A., Hill A., 1975). Early diagnosis is the most important factor in preventing irreversible lung damage (Schatz M., Patterson R., 1983).

Aetiologic Agents

A variety of agents have been implicated as causing HP acquired from airconditioning systems. Generally, specific identification is unnecessary in achieving a diagnosis. Challenge studies and immunologic tests can be performed on heterogeneous solutions of environmental material, however, the identification of the allergen is helpful in obtaining information for epidemiological purposes.

The principal allergens are thermophilic Actinomycetes including Micropolyspora faeni, Thermoactinomyces vulgaris, T. sacchari, T. viridis, T. candidus and Saccharomonospora species. These ubiquitous, saprophytic bacteria are frequently isolated from airconditioning apparatus (Schatz M., Patterson R., 1983). Other organisms have also been implicated in HP: amoebae, including Vahlkampfia sp, Naegleria gruberi, Enchinamoeba exundans (Edwards J.H., 1980) mites including Dermatophagoides pteronyssinus; Bacillus subtilis

(Parrot W.F., Blyth W., 1980); and the fungi, Cephalosporium sp (Patterson R., Fink J.N., Roberts M., 1978) and Aspergillus fumigatus (Van Assendelft A., Forsén K.O., Keskinen H., 1979).

It is not unusual for multiple precipitin bands to be evident in immunodiffusion studies using the patients' sera and environmentally obtained extracts. This suggests that the patient is susceptible to more than one allergen. Because of the complexity of the antigenic material, in many cases a definitive aetiologic agent is not identified (Marinkovich V.A., Novey H.S., 1983). Cross reactivity between humidifier extracts obtained from separate outbreaks of HP has been documented (Edwards J.H., 1980).

Pathology and Pathogenesis

All people are exposed to a wide variety of antigenic substances, however, only a few react in an adverse fashion. A subject's response depends mainly on the immunologic reactivity, but, the type of material inhaled, the size, density and quantity of particles are also important. Particle shape and size is important in determining whether the antigen reaches the respiratory bronchioles and alveoli where it may evoke an immunologic reaction (see Chapter 4). The intensity of exposure is a significant factor in clinical presentation. High level exposure of a short duration may result in an acute interstitial pneumonitis which is reversible, whereas chronic low level exposure may lead to the

insidious development of symptoms and irreversible lung damage (Schlueter D.P., 1974). The size of the particles spread by airconditioning systems and causing HP has not been recorded. In the case of 'farmers' lung', an HP caused by the inhalation of thermophilic Actinomycetes from mouldy hay, up to "750,000 fungal spores per minute" may be deposited in the lungs (Schlueter D.P., 1974).

It is not uncommon for patients with HP, particularly those with the insidious form, to have lung biopsies performed in order to confirm the diagnosis by histologic analysis. Whilst pathologic changes are not pathognomonic for HP, they are suggestive of the disease. The histologic appearance of the lungs depends on the stage of the disease (Schlueter D.P., 1974).

The following are histologic reports of typical lung biopsies performed on two patients, one with the acute form and the other with the insidious form of HP:

Acute form: the lung biopsy showed an infiltration of alveolar septae by lymphocytes, plasma cells and occasionally histiocytes. Focal non-caseating granulomas were also present.

Insidious from: the lung biopsy showed an interstitial infiltrate comprising lymphocytes, plasma cells, a few neutrophils, eosinophils and fibroblasts. The alveolar spaces contained an exudate of alveolar macrophages, neutrophils and eosinophils (Fink J.N., Banaszak E.F., Barboriak J.J., 1976).

The evidence from experimental and human studies strongly suggests a Type III (Arthus reaction) immune complex pathogenesis for the early disease stage. The granulomatous formation that occurs late in the disease is a Type IV delayed hypersensitivity reaction (Robbins S.L., Cotran R.S., Kumar V., 1984).

Environmental investigation

The airconditioning system

The investigation of HP requires a close examination of the patients' environment for the origin of the disease. Although this site is most likely to be the workplace there have been reports of HP having been acquired from airconditioners situated in the home or in the car (Marinkovich V.A., Novey H.S., 1983). A detailed study of the airconditioning system is required with particular attention being paid to potentially allergenic material.

The most common source of allergenic material is humidifier water and several litres should be collected for laboratory analysis. Sludge accumulating on the baffle plates is also frequently implicated in HP. Other sources include ductwork, false ceilings, drip trays, evaporative cooling systems, air washers, filters and baffle plates (ACOA Report, 1984; Editorial, 1984).

Air sampling has occasionally been performed to determine the fungal and bacterial content of the air (Parrot W.F., Blyth W., 1980).

Microbiological analysis

Culture for microorganisms is performed in order to identify specific allergens. Once in pure culture, the organisms can be tested in gel diffusion studies or killed and used as challenge material.

Thermophilic Actinomycetes have been implicated in many cases of HP. They can be isolated on nutrient agar, half strength nutrient agar or tripticase soy agar incubated at 50°C. Identification can be achieved through; cultural and microscopic morphology on various media; biochemical tests, including decomposition of tyrosine, xanthine, hypoxanthine, gelatin, casein, esculin and arbutin; rapid thin-layer chromatography (Kurup V.P., Fink J.N., 1975).

Fungi can be cultured on Sabouraud's agar or corn meal agar incubated at room temperature. Nutrient agar incubated at room temperature and 37°C is satisfactory for the growth of bacteria (although selective agents may be required). Protozoa, including amoebae, can be isolated by inoculating the specimen on to lawn plates of Klebsiella aerogenes grown on non-nutrient agar. The plates are incubated at room temperature for up to 10 days and the protozoa are separated by extracting the agar with 1% phenol, concentrating and dialysing (Edwards J.H., 1980).

Immunologic tests

Demonstration of serum precipitin bands to an extract of environmental material indicates that an individual has

been exposed to an allergen. This can be useful in epidemiologic studies and also in identifying the antigen. The identification of asymptomatic individuals with precipitins is important as continued exposure may precipitate HP (Pickering C.A.C., Moore W.K.S., Lacey J., 1976).

Gel diffusion techniques are the most commonly used methods in determining exposure to an allergen. The initial step is to prepare the antigen. Environmental material, such as humidifier water, baffle platesludge and filter debris is concentrated through filtration then air dialysed (although centrifugation followed by sonication is also reliable). Extracts from pure isolates can be prepared by culture in a broth medium such as casein hydrolysate for fungi and a nutrient broth for bacteria. The broth is then dialysed and the cells extracted by a Hughes Press (Edwards J.H., 1980).

The gel diffusion plates are prepared using 1% purified agar in a buffered solution eg. M^{c}Ilraines' citrate-phosphate buffer or barbitone buffer. A central well of 10mm is cut into the agar with 4mm diameter peripheral wells in a hexagonal arrangement 10mm centre to centre apart. The test serum is placed in the central well and extracts are placed in the surrounding wells. Plates are incubated at room temperature for two days (Edwards J.H., 1980; Hudson L., Hay F.C., 1976).

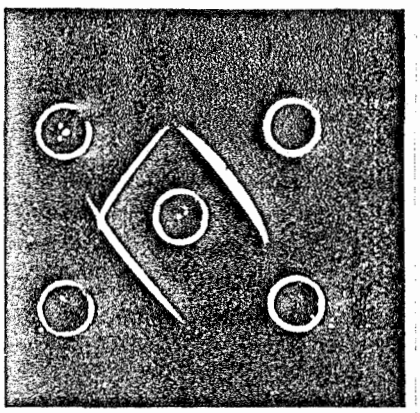
Following incubation the plates can be read directly using an oblique light source or they can be stained with a protein dye, eg. Coumassie blue (Hudson L., Hay F.C., 1976)

A precipitin band forms where the antigen and antibody meet. Multiple lines of precipitation will be present if the antigen and antibody contain several molecular species (Hudson L., Hay F.C., 1976).

Figure 2

Gel diffusion plate

(Hudson L., Hay F.C., 1976)



Antigen - centre

Positive serum - bottom left, top left, top right

Negative serum - bottom right

ELISA techniques have been employed to demonstrate exposure to environmental material. Good correlation has been observed between the results of gel diffusion and ELISA tests (Cockcroft A., Edwards J., Bevan C., 1981).

Epidemiology

Airconditioning systems provide a perfect environment for the growth of a range of microorganisms including moulds, fungi, amoebae, bacteria and nematodes (ACOA Report, 1984). This material comprises the allergen that causes disease amongst the buildings' occupants. An examination of a typical study demonstrates how this occurs.

During 1968 and subsequent years, nine men employed in a large printing works in Britain, were displaying recurrent respiratory symptoms during the winter months. Complaints were most common after weekends or other absences. Investigators considered the symptoms to be suggestive of an allergic disease with the suspicion of an airborne allergen.

Between October and May, the ventilation system used water from the town supply for the humidifier. Recirculation was necessary to avoid waste and a biocide (Panacide, BDH), recommended by the Printing Industry Research Association, was added to the water to control bacterial growth. In spite of this there was considerable slime growth on the baffle plates through which the air left the humidification chamber. In summer, cooling was achieved through the use of bore-hole water which was run to waste after being sprayed into the humidifier once only.

The air-intake was a variable mixture of fresh and recirculated air. The proportion of fresh air rose to 50% during the cooler months and fell to 2% in summer (Pickering

C.A.C., Moore W.K.S., Lacey J., 1976).

This case is one of a number involving printing works (Panott W.F., Blyth W., 1980; Edwards J.H., 1980; Friend J.A.R., Gaddie J., Palmer K.N.V., 1977). It appears that paper, rayon fly and off-set powders consisting of disaccharides and polysaccharides encourages microbial growth. In the case study outlined, recirculated air was used particularly in the cooler months when symptoms were most noticeable. This most probably provided the vehicle for the printing waste to contaminate the airconditioning system. Recirculated water appears to have enhanced the degree of contamination despite the use of a biocide.

Reservoir

Organic dust contaminating the airconditioning system provides a biomass for microorganisms entering from the air and water supplies (Marinkovich V.A., Novey H.S., 1983). Multiplication of these organisms occurs particularly in the humidifier water but also on the baffle plates. Contamination of ducts and false ceilings with dust can also provide a source of allergenic material.

Transmission

It is not always possible to prove that the mode of transmission in outbreaks of HP is airborne, however, it is apparent that this is most likely. Organisms have been isolated from air samples to which precipitin bands have been demonstrated in symptomatic patients (Parrot W.F., Blyth W., 1980).

Antigenic material has been obtained from humidifier water that has not been recovered by culture (Marinkovich V.A., Novey H.S., 1983). As most air sampling techniques rely on the isolation of microorganisms, it is possible the allergen has been missed. Liquid impingement samples may assist, however, large volumes of air would be required so that the allergen could be concentrated and thus the mode of transmission demonstrated.

Further evidence for an airborne route of transmission is suggested by reproduction of symptoms with respiratory challenge (Fink J.N., Banaszak E.F., Barboriak J.J., 1976).

Susceptible hosts

It is not understood why only 3-16% of people exposed to allergens develop HP (Arnow P.M., Fink J.N., Schlueter D.P., 1978). Genetic studies have not detected consistent differences in the frequency of specific HLA antigens between patients and control populations (Robbins S.L., Cotran R.S., Kumar V., 1984). It is also unclear why some subjects respond to the allergen by developing symptoms of the acute disease while others acquire the insidious form of HP.

It has been suggested that the development of precipitins is the first stage in HP and that continued exposure to the allergen may precipitate disease (Pickering C.A.C., Moore W.K.S., Lacey J., 1976). Certainly, surveys of the general population have failed to show precipitins whilst up to 75% of people exposed to allergens develop them (Cockcroft A., Edwards J., Bevan C., 1981).

No correlation has been made between smoking and susceptibility to HP (Edwards J.H., 1980). People prone to allergies are not predisposed to the disease (ACOA Report, 1984).

Surveying for hypersensitivity pneumonitis

Following the index case(s) which alerts the authorities to the possibility of HP, a questionnaire survey can be useful in identifying other workers with symptoms. Suspect cases can then be defined from this information (Arnow P.M., Fink J.N., Schlueter D.P., 1978).

A questionnaire assists in the epidemiology of the disease by determining the pattern of symptoms, the work areas of those affected, smoking habits etc. Thus a typical questionnaire may include: length of employment; specific work area; work hours; symptoms noticed; period over which symptoms experienced; sick leave; medical history (ACOA Report, 1984).

Control

Methods that have been recommended to control HP include the following:

1. Better input filters to reduce the amount of dust and microbial material that is drawn into the system. Cloth or fibre filters are unsatisfactory. A pre-filter combined with an electrostatic filter enable particles less than 5um to be removed. Additional filters may be needed at air supply vents.

2. Steam injection humidifiers should be used, thus eliminating the need for a recirculating water system.

3. Frequent cleaning to reduce the build-up of micro-organisms. Cleaning should be performed every 2-3 weeks.

4. Regular filter replacement.

5. The addition of chemicals (disinfectants and biocides) is recommended by some workers but not all, as chemicals may spread to the building and harm the occupants.

6. Ultraviolet light at the air intake.

7. Improved design of the system with the elimination of conditions favourable to microbial growth. The system should allow easy access for cleaning and maintenance (ACOA Report, 1984; Marinkovich V.A., Novey H.S., 1983).

Decontamination of an airconditioning system requires careful consideration. The source of contamination needs to be identified before remedial actions can be taken. Ultimately it may be necessary to install a different system (Cockcroft A., Edwards J., Bevan C., 1981).

SUMMARY

Allergic reactions to organic dust spread by air-conditioning systems can be of either an acute or an insidious nature. Diagnosis depends upon close attention to the patient's history, particularly exposure to conditioned air. Radiologic, pathologic and immunologic tests are available to assist in diagnosis. Gel diffusion tests can identify asymptomatic subjects with evidence of exposure to allergenic material. These people may progress to HP.

The identity of the allergen may be any of a number of microorganisms although thermophilic Actinomycetes are the most common.

Prevention of HP can be achieved by improving the design of airconditioning systems, regular cleaning and the use of efficient filters.

A greater understanding of the pathogenic mechanisms of HP as well as the identification of the responsible allergens should improve the diagnosis and investigation of HP. Rapid diagnosis is important in order to avoid the irreversible lung damage that can occur in the insidious form of the disease.

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CONCLUSION

The review of material covered in this thesis has exposed certain factors that need to be addressed in an overall perspective. Four areas are considered.

Awareness

There is no doubt that the massive publicity surrounding the 1976 outbreak of LD in Philadelphia, contributed to the increased diagnosis of the disease. Informed medical officers and scientists were able to direct their attention towards this diagnosis. HP has not attracted so much interest and may be misdiagnosed as a result. A greater appreciation of the epidemiologic aspects of HP and LD will also aid in their prevention.

Whilst engineering and medical personnel can be further informed regarding these diseases, a major breakthrough in control will be achieved by educating the public. If those working in airconditioned buildings are made aware of various aspects of LD and HP, then they may assist diagnosis through early recognition of the symptoms.

Prevention

The principal site of contamination by LD bacilli (LDB) is the cooling tower. These structures are situated on the top of buildings and are exposed to the environment. Control of LDB in cooling tower water represents the first step in prevention. It is not clear if contamination could be eliminated through design alteration, but this could be

determined. At present, biocide treatment is the recommended form of control, however, there is a need to improve the efficiency and safety of these chemicals.

Home humidifying units may represent a significant reservoir for LDB and other microorganisms. The public need to be educated with regard to the hazards that can occur if the systems are inadequately maintained allowing microbial contamination. Both LD and HP can be contracted from this situation.

Water recycling humidifiers such as the spray humidifier are the major sources of HP allergens. Steam humidifiers do not become contaminated and are recommended.

Efficient filtration at the air inlet will restrict the number of microorganisms in contact with airconditioning apparatus. Such filtration should consist of a coarse prefilter with a second filter capable of removing particles of 0.01um in size. An electrostatic filter, although expensive, would be appropriate. UV filtration may be useful in hospital rooms to prevent cross infection, but its use in the airconditioning system is doubtful.

Workers in buildings are exposed to a concentration of microorganisms that they would not experience in the open air. This factor increases their susceptibility to disease due to the inoculum challenge that they may experience. If airchange rates were increased (without causing uncomfortable draughts), then the disease threshold may be avoided.

It has been reported that a decrease in relative humidity to below 30% can increase the likelihood of infection. Many airconditioning systems in Australia do not incorporate a humidifier because the air is generally regarded as being sufficiently moist. If, however, humidity did fall below 30% the facility would not be available to raise it. This may result in irritation and infection, particularly to the mucosal surfaces. It is not known if this would be a predisposing factor to either LD or HP but it could be determined using animal models and possibly through retrospective or prospective studies.

Reclaiming energy from the airconditioning system may present a significant hazard if not performed with caution. Heat exchangers appear to be perfectly safe, but recirculating air and water can be a dangerous practice. In the printing industry, for instance, it has been reported that paper waste has contaminated humidifiers during the recycling of air thus providing a nutrient source for the multiplication of microorganisms. If air and water are to be recycled the most stringent conditions must be set to ensure complete safety.

Printing and textile industries have been involved in multiple outbreaks of HP while a similar trend has been noticed in hospitals and mental institutions with LD. Certain individuals are particularly susceptible to LD such as the aged, smokers and renal transplant patients. These observations may assist in control and investigation of LD and HP.

Further studies regarding predisposition to these diseases may reveal valuable information that can identify people at risk of acquiring them.

Probably the most significant factor that will influence the control of microbiological contamination in airconditioning systems is improvement of their design. The first step is to improve the accessibility so that effective cleaning can be achieved followed by a move to eliminate water-containing apparatus.

Guidelines for investigating airconditioning systems

In the event of a disease presumed to have been acquired from an airconditioning system, the building concerned will require extensive investigation. The following guidelines are offered for conducting this study, however, it must be understood that each investigation will require an individual approach.

1. The patient's history: is obtained along with a presumptive diagnosis. It should be possible to differentiate between HP and LD at this stage.

2. Examination of the airconditioning system: is performed preferably in conjunction with the site engineer.
   N.B. The hot water system should also be examined if LD is suspected.

   Consider the following:

   design

   airflow (including the air intake)

water-containing apparatus

condition of the system

the maintenance procedures and standards

filters, type and condition

recycling of air and water

use of the building (eg. printing works, hospital)

3. Specimen collection: is performed early in the investigation as the system will need to be closed down, all water drained, and cleaning initiated.

Collect:

water from all sites, including source water

dust and organic material

blood specimens from all workers

airsampling, if the investigators have the equipment and expertise

4. A questionnaire survey: is conducted.

5. Laboratory investigations:

LD - culture of water specimens for LDB

direct fluorescent antibody tests on water specimens for LDB

serology for antibodies (a convalescent serum specimen will be required).

HP - culture of water and other environmental specimens for thermophilic Actinomycetes, amoebae, bacteria, fungi

microscopy for mites, nematodes, algae, pollen, protozoa, other

immunodiffusion studies for antigen and precipitin band detection

Store all material for retrospective analysis.

6. Report/consider further action

All well-equipped laboratories should be able to perform these investigations.

LD Research

The indirect fluorescent antibody test (IFAT) performed on serum can be diagnostically useful when IgM antibodies are detected in a titre >16, however, there is no doubt that more rapid techniques are needed for the diagnosis of LD. There are two avenues worth pursuing, i) the detection of urinary antigen, ii) the use of monoclonal antisera in direct fluorescent antibody (DFA) techniques so as to overcome problems with nonspecific fluorescence.

One major disadvantage of the IFAT is that all serogroups of LDB must be tested before the serum can be regarded as negative. At present at least 13 serogroups are known. Study should be directed towards finding a common antigen. A less satisfactory alternative would be to determine the serogroups of LDB present in the area by examining local cooling towers and hot water systems. The prevalent strains of LDB could then be used in the IFAT.

The relationship between LDB and other microorganisms bears closer attention. Some degree of symbiosis appears to occur between LDB and amoebae and also with blue-green algae. Rust and possibly other inorganic material may also act as growth promotants. The survival of LDB in treated

cooling towers may be linked with these factors.

Evidence indicates that LDB produces a toxin, albeit different to that of other gram negative organisms. Further research is required to determine more of the pathogenesis of this disease particularly with respect to toxin production.

The suggestion has been made that Pontiac fever is a type of HP with the allergen being amoebae containing ingested LDB. This may explain certain differences between this disease and LDB. Environmental material and serum from affected individuals will be required in order to pursue this avenue of investigation.

Airsampling has not been successful in isolating LDB and yet useful information may be obtained if this were possible. One of the problems is that LDB are difficult to grow on artificial media and as airborne organisms may be damaged anyway, the difficulties are compounded. The use of an Anderson air sampler is not indicated in this circumstance but possibly a liquid impinger would be satisfactory. The recovery fluid would contain an increased concentration of sugar and also ferric pyrophosphate and L-cysteine. A selective medium would then be used after a suitable recovery period. Alternatively a DFA test could be performed on the liquid impinger fluid, although the viability of organisms would not be determined.

HP Research

A major factor hindering research into HP has been the use of inconsistent and confusing nomenclature. In this thesis the terminology used by Banazak, who first described the disease, is proposed. The terms HP acute form and HP insidious form are both accurate and descriptive. If a definition of the two forms of HP could be agreed upon, then similarities and deviations from this reference point would be evident.

There is relatively little known about the disease, HP. Fundamental information has yet to be obtained concerning the nature of the allergen, predisposing factors, the significance of precipitin bands and the mode of transmission. The gel diffusion test is presently employed to determine exposure to the allergen, however, this is a relatively crude technique. Although it may turn out to be sufficiently sensitive for this application, the development of more sophisticated procedures such as ELISA and electrophoresis should be encouraged in an effort to improve knowledge.

The number of cases of HP occurring in printing works prompts further consideration. It should be easy to determine if paper-waste is contaminating the airconditioning system and encouraging microbial growth. A detailed study of printing works may be a valuable place in which to commence research into HP.

Hypersensitivity pneumonitis and Legionnaires' disease are relatively uncommon diseases. HP affects apparently healthy people, but if diagnosed early, serious consequences can be avoided. LD rarely affects healthy individuals and should not cause alarm to the general population. There is a possibility that other diseases will be associated with microbiologically contaminated airconditioning systems but it is unlikely that they will be life-threatening or they would otherwise have already been documented.

The indications are that HP and LD could be substantially prevented through instituting the recommendations included in this thesis. Disease amongst some susceptible hosts such as renal transplant patients, however, may be unavoidable The factors that will control these diseases in the forseeable future are awareness, preventative techniques and further research. In the event of disease occurring, the guidelines presented here will assist in diagnosis.

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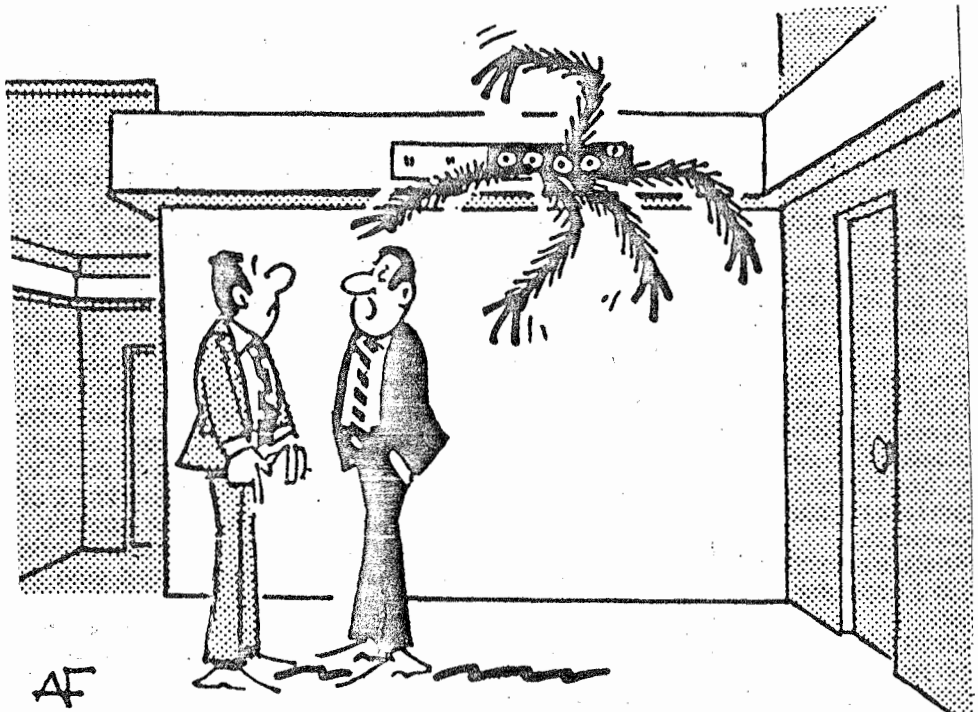
A FINAL COMMENT.....



"WHAT'S ALL THIS NONSENSE ABOUT SOME KIND OF FUNGI OR BACTERIA IN THE VENTILATING SYSTEM?"

.....Courtesy Victorian Public Service Association.